

THE LYMPHATICS SYSTEM OF THE GUINEA-PIG

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NINETEEN FIGURES

INTRODUCTION

This investigation of the lymphatic system of the adult male guinea-pig was entirely macroscopic. Owing to the technical difficulties the lymphatics of certain organs of the body such as the stomach were not injected successfully. Yet, in the more general sense, apart from the minor details, the fundamental plan of the system of the guinea-pig was revealed.

The literature on the anatomy of this animal is very limited but the anatomy of the bones and the muscles by Alezais, '98-'02 has materially assisted me in this study.

The method of investigation was as follows: The animal was killed with ether and while it was still warm, injections were made by means of the ordinary hypodermic syringe provided with a fine glass cannula. The animal injected was then carefully dissected in the fresh or after temporary preservation. The needle must be of very fine caliber, indeed, in order to obtain a successful injection of the lymphatics of the guinea pig. The finest hypodermic needles obtainable in the market are too large, for their use usually causes a large area of the extravasation without filling any lymphatic vessels. Such a needle was, however, improved by fusing on to it a fine glass cannula, the tip of which was drawn out into a short but very delicate capillary tube. Among the various injection masses that were tested, Gerota's Prussian blue and diluted India ink gave the most satisfactory results.

This investigation is based upon experiments on about 30 male guinea-pigs, which after the injection, were carefully dissected with the aid of a watch-maker's lens.

Injection of the cutaneous lymphatics offered no little difficulty when I attempted to work from the outer surface of the skin, for the needle either penetrated too deep or too superficially. This difficulty was overcome by reflecting a small flap of skin by a crescentic incision and inserting the needle from the under surface. However injection must be made very slowly and under low pressure.

Working with the mesenteric nodes, it was found that when any of them were forcibly distended with injection fluid in order to inject the lymphatics backward to the loop of the intestine, the veins received retrograde injection instead as mentioned previously by Meyer, '14.

For the purpose of description the body is divided into the following regions: the head and neck, the upper extremity, the lower extremity, the thorax and the abdomen. The lymph-glands of the entire system are first described with reference to their average size, general shape, location and their afferent and efferent vessels. This is followed by the consideration of the lymphatic vessels of each region. The names of the glands are adopted, as far as possible, from the corresponding glands of the human lymphatics, but there are a few glands, which because of their closer similarity to those of cattle, are named after the work of Baum, '12. However, since some glands are situated quite differently in the guinea-pig from corresponding glands in man and other animals, it seemed better to adopt new names than to rigidly adhere to a given nomenclature. These glands were the following:

A. Infra-mandibular node. From the efferent and afferent vessels, the node corresponds to the submental node. It lies behind the mandible and the term inframandibular node seems quite appropriate.

B. Dorsal and ventral pre-scapular node. These nodes correspond to the deep cervical nodes but since they lie at the level of the cephalic border of the scapula the term pre-scapular seems the more appropriate.

C. Retro-scapular node. This belongs to the group of axillary nodes but lies wholly outside of the axillary space so that the term axillary node could only express a rough correspondence. Since it is more closely in relation with the caudal border of the scapula, the term retro-scapular seemed quite justifiable.

D. Abdomino-inguinal and inguinal nodes. These nodes correspond to superficial and deep inguinal nodes respectively but their relation is so disturbed by the peculiar position of the hind limb that what might be the superficial inguinal node is pushed out of place and lies on the abdominal wall under cover of the tensor fascia lata, so that the term abdomino-inguinal is suggestive both of position and correspondence.

The term inguinal node instead of deep inguinal is used because there is no superficial inguinal node. Consequently the adjectives 'superficial and deep' are superfluous and the term inguinal is employed to indicate a gland homologous with the deep inguinal node.¹

I. THE LYMPH GLANDS

Lymphoglandula parotidea (figs. 1, 15.) Position and size. The gland is found caudal to the base of the auricle, on the dorso-caudal border of masseter muscle and on the external maxillary vein. It is covered by the cephalic portion of the platysma and skin and is imbedded in the parotid gland. Unless injected, it is very difficult to recognize it. It is flat and ovoid in shape, measuring 4 by 3 by 2 mm.

Afferent vessels. The chief afferents of the auricle terminate in this node.

Efferent vessels. One or two vessels from the node follow the external maxillary vein into the substance of the parotid gland and terminate in the submaxillary node which is placed at the junction of the external and internal maxillary vein and covered partly by the submaxillary salivary gland.

Lymphoglandula inframandibularis (figs. 1, 2) This gland is located on the ventral surface of the anterior belly of the digastric muscle in close proximity to that on the opposite side. These nodes are usually placed transversely and one of them may be found quite behind (caudal to) the other. They are covered by the platysma and the skin. They are round and flat, 5 to 6 mm. in diameter and 2 mm. in thickness.

¹ A supernumerary adrenal body was often found near the renal vessels. This was identified histologically.

It is not, however, infrequent to find one of them considerably larger and more elongated and measuring 9 mm. in length.

Afferent vessels. The lymphatics of the lower lip and those of the anterior two-thirds of the tongue terminate in this node.

Efferent vessels. One or two vessels pass transversely between the sterno-hyoid and digastric muscles where they join with the collectors from the posterior part of the tongue. These conjoined vessels are directed caudal on the superficial surfaces of the sterno-hyoid and scalenus anterior and terminate in the deep cervical glands.

Lymphoglandula submaxillaris (figs. 1, 2, 15) Situation and size. This node which is entirely hidden by the parotid and submaxillary

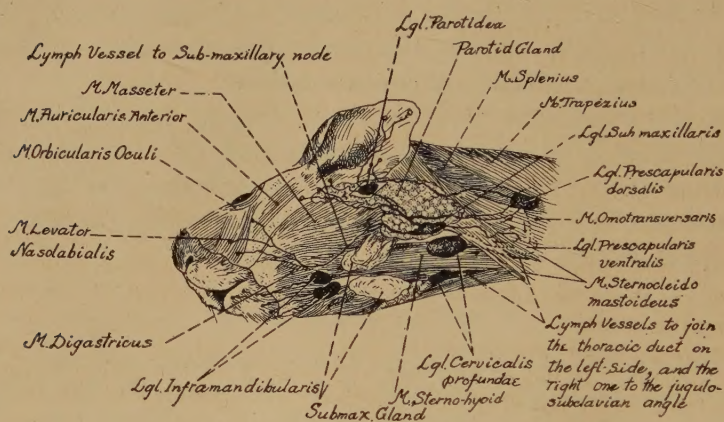


Fig. 1 Superficial lymphatics of the neck

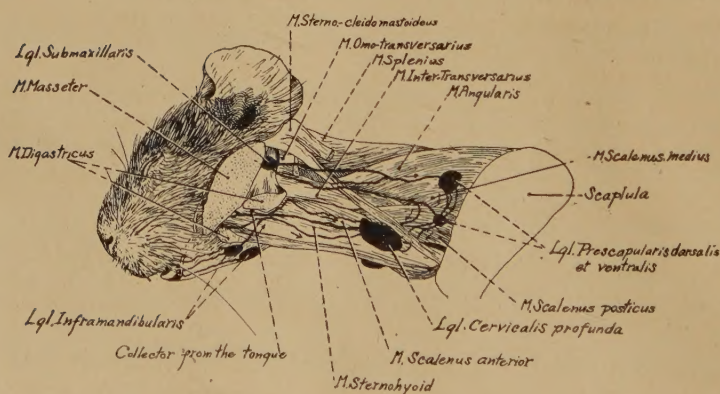


Fig. 2 Deep lymphatics of the neck

salivary glands lies at the junction of external and internal maxillary veins and is covered by the platysma. It is an irregularly shaped gland measuring about 4 mm. in diameter and 2 mm. in thickness. It is often represented by two glands; one medial to the other; but in very close relation to each other and separated only by the small portion of the submaxillary salivary gland and a part of the veins. When double these glands are connected with a number of short anastomosing lymph vessels.

Afferent vessels. The vessels from a small portion of the auricle, from the frontal cutaneous area around the eye-lids, and efferents of the parotid node terminate in this node.

Efferent vessels. Two or three vessels from this node follow the external jugular vein. At the lower border of the sternocleidomastoids, the vessels take three different directions. One passes over the dorsal and the other over the ventral and dorsal prescapular nodes. Another vessel passes directly into the deep cervical nodes.

Lymphoglandula prescapularis dorsalis (figs. 1, 2, 15). This node lies on the angularis muscle cephalad to the scapula and is covered by the omotransversarius and trapezius muscles. It is ovoid in shape, measuring 5 to 7 mm. long, 4 mm. wide and 3 mm. thick.

Practically all the cutaneous lymphatics of the neck and those from the skin covering the cephalic portion of the scapula and the efferents from the parotid and submaxillary nodes terminate in this node.

Two or three vessels are directed ventrally to end in the ventral prescapular node.

Lymphoglandula prescapularis ventralis (figs. 1, 2). This gland is rarely absent. It lies at the root of the neck on the scalenus medius, and is covered by the clavicular portion of the sternocleidomastoid and platysma muscles. It is round and flat, measuring 4 mm. in diameter and 2 mm. in thickness.

Two or three vessels from the dorsal prescapular and one or two from the submaxillary nodes terminate in this node.

Usually two vessels pass ventro-cephalad under the sternocleidomastoid muscles to the deep cervical nodes.

Lymphoglandula cervicalis profunda (figs. 1, 2). This gland lies at the bottom of the V-shaped space formed by the sternocleidomastoid laterally, and the sternohyoid medially and is in contact with the trachea and anterior scalenus muscles lying in close relation to the internal jugular vein and the carotid artery. It is the largest node in the region of the head and neck. It is ovoid in shape and flattened, being 8 to 11 mm. long, 6 mm. wide and 3 mm. thick.

All lymphatics from the head and neck finally terminate in this node. It receives the collectors from the tongue and the efferent vessels from the submandibular, submaxillary and ventral prescapular nodes.

The efferent vessel from this node is the largest lymph vessel in the neck. It is 12 to 15 mm. long, distinctly sacculated and closely follows the internal jugular vein, lying between the sternocleidomastoid

and the anterior scalenus muscles. At the root of the neck it terminates on the left side at the cervical bend of the thoracic duct and on the right side, at the junction of the internal and external jugular veins.

2. The lymphglands of the anterior extremity

Lymphoglandula retro-scapularis (figs. 4, 5). This node is located on the outer surface of the latissimus dorsi at the angle formed by the caudal border of infraspinatus and the dorsal border of the triceps brachialis. It is imbedded in a pad of fat and covered by the panniculus carnosus and the skin. It is ovoid in shape and flat, 9 mm. long, 4 mm. wide and 2 mm. thick.

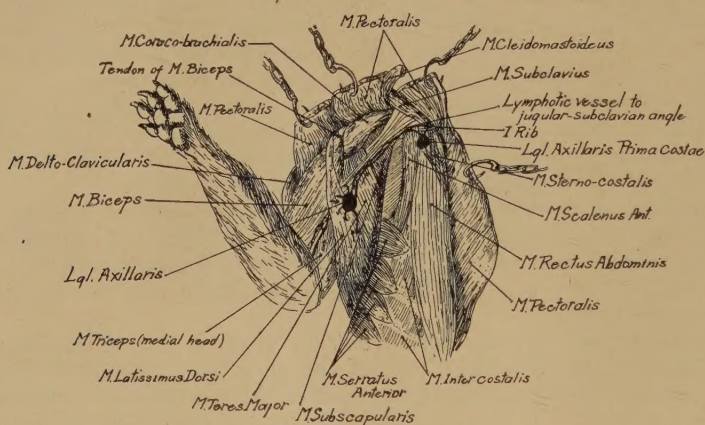


Fig. 3 Lymphatics of the axilla

The lymphatics of the outer surface of the anterior limb, and the dorsal and lateral cutaneous lymphatics of the thorax terminate in this node.

Two or five vessels from this gland penetrate the latissimus dorsi and terminate in the lymphoglandula axillaris.

Lymphoglandula axillaris (figs. 3, 17). This gland lies on the medial surface of the teres major at the angle formed by the axillary artery and the tendinous insertion of the latissimus dorsi. Medially it is in relation with the scalenus anterior. It is a circular node 5 to 7 mm. in diameter and 2 mm. thick.

It receives efferent vessels from the retro-scapular node and the inner cutaneous lymphatics from the pectoral region also terminate in it.

The large single trunk which follows the axillary artery is interrupted by the lymphoglandula axillaris prima costae. However, it is not infrequent to find this trunk dividing near the latter node into two

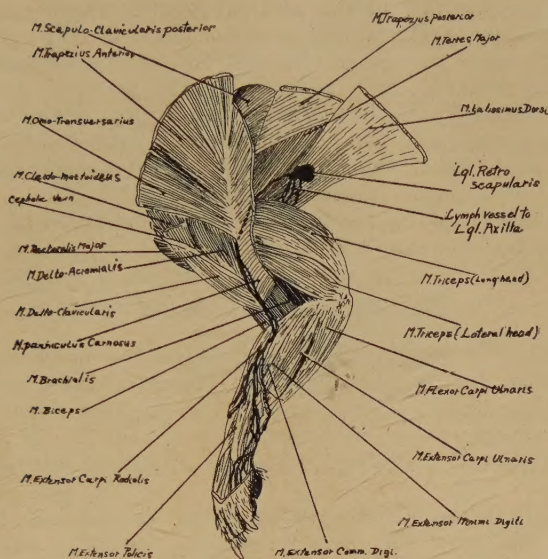


Fig. 4 Lymphatics of the fore limb

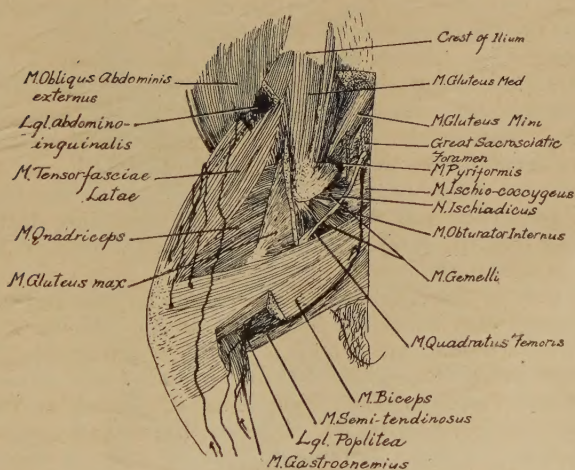


Fig. 5 Lymphatics of the thigh

vessels, one of which terminates in this node and the other at the junction of the external jugular and subclavian veins.

Lymphoglandula axillaris prima costae (figs. 3, 17). This node is found on the outer surface of the first rib, at the termination of the axillary blood vessels, being covered by the pectoral muscles. It is circular and about 5 mm. in diameter and 2 mm. in thickness.

The efferent vessels of the lymphoglandula retro-scapularis and very often some efferents of the lymphoglandulae tracheobroncheales and lymphoglandula axillaris end in this node.

A large single trunk leaves the node and terminates at the junction of the subclavian and external jugular veins.

3. Lymphatics of the lower extremity

Lymphoglandula poplitea (figs. 5, 7, 15). The node lies imbedded in the popliteal fat, in the triangular space bounded by the biceps femoris, semitendinosus and gastrocnemius muscles. It is spherical in form and about 2 to 3 mm. in diameter.

The lymphatics of the lateral surface and dorsum of the foot terminate in it.

The majority of the efferent vessels follow the popliteal blood vessels where they join with the deep lymphatic vessels of the hind limb. However, there is one other efferent vessel which passes along the posterioro-caudal border of biceps the femoris. Near the origin of the latter it crosses the outer surface of the muscle and soon passes under the gluteus maximus to accompany the ischiadic nerve with which it enters the pelvic cavity through the foramen ischiadicum majus. In the pelvis it lies close to the median line on the anterior surface of the sacrum and finally terminates in the hypogastric node.

Lymphoglandulae abdomino-inguinales (figs. 5, 6, 7, 15). These glands are found along the course of the superficial circumflex iliac vessels between the obliquus abdominis externus and tensor fasciae lata muscle. This group consists of 2 to 4 nodes of varying size, the largest of which reaches about 7 mm. in diameter.

All cutaneous lymphatics of the abdomen and the hip, the lymphatics of the outer and inner surfaces of the thigh, those from the scrotum and from a small portion of the lateral part of the hind leg terminate in this node.

The majority of the efferent vessels accompany the superficial circumflex iliac vessels and terminate in the inguinal nodes but a small vessel passes through the femoral ring to end in the iliac node.

Lymphoglandulae inguinales (figs. 6, 7). This pair of nodes is found on the femoral vessels, close to the femoral opening and is composed of a medial and a lateral node. The medial node is irregular in shape measuring about 5 mm. in diameter and 2 mm. in thickness. The lateral node is the smaller of the two. It is circular and measures about 3 to 4 mm. in length and 1 mm. in thickness.

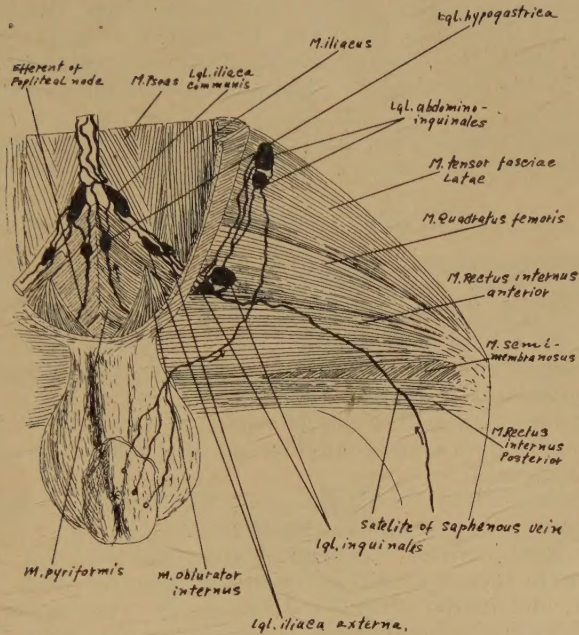


Fig. 6 Lymphatics of the femoral and pelvic regions



Fig. 7 Lymphatics of the abdominal and femoral regions

The efferents from the popliteal nodes, the deep lymphatics of the hind limb and those accompanying the saphenous artery terminate in the medial, while the efferent vessels of the abdomino-inguinal nodes terminate in the lateral node. These two nodes are in communication with each other.

A number of vessels pass through the inguinal canal and terminate either in the external iliac or in the common iliac nodes.

4. The Lymph glands of the abdomen

Lymphoglandula iliaca externa (figs. 6, 7). This is a very inconstant gland located when present on the external iliac vessels. It is quite round and 3 mm. in diameter and 2 mm. in thickness. It is interpolated in the course of the efferent vessels coming from the inguinal nodes but sometimes also receives afferents from the bladder.

Lymphoglandula hypogastrica (figs. 6, 7, 10, 16) This is a small but constant gland lying in the pelvic cavity at the origin of the hypogastric artery. It is a small circular gland 2 mm. in diameter and 1 mm. in thickness.

The lymphatics of the bladder and the seminal vesicle and the efferent vessel from the popliteal node terminate in it.

An inconstant number of efferents cross the common iliac vessels to terminate in the common iliac node while certain other vessels pass toward the abdominal aorta, contributing to the formation of the lymphatic plexus around this vessel and the inferior vena cava.

Lymphoglandula iliaca communis (figs. 6, 7, 10, 16). This node is placed at the angle of abdominal aorta and the common iliac artery. Indeed it is not infrequent to find it lying entirely on the outer side of the common iliac vessels. It is fusiform in shape, 7 mm. long, 3 mm. wide and 2 mm. thick.

The entire lymph stream of the lower extremity and from the pelvic cavity reaches it. Vessels from the bladder and from the pelvis together with the efferent vessels of the inguinal, the external iliac and the hypogastric nodes terminate here.

As soon as the efferent vessels from this node reach the side of the great abdominal vessels, they form a very rich plexus which entirely surrounds these vessels. Their final destination is the cisterna chyli, but before reaching it they are interrupted by small 'Schalt-drüsen' scattered in the course of the plexus.

Lymphoglandulae mesentericae intestinales (fig. 9). A large number of lymph nodes of varying size are found between the two layers of the mesentery. Among these is one large node which lies on the main trunk of the mesenteric vessels. This node receives the entire lymph stream of the intestine. The rest of the nodes are scattered on the peripheral branches of the same vessels, constituting glands for different segments of the intestine. These latter glands are quite variable. Hence the description of a single type of arrangement can not be entirely satisfactory. Nevertheless, there is a certain prevailing arrangement which is considered here as typical.

The following glands constitute the lymphoglandulae mesentericae intestinales:

1. The lymphoglandula colicae descendents.
2. The lymphoglandula colicae transversalis.
3. The lymphoglandula colicae ascendents.
4. The lymphoglandula intestini tenuis.
5. Lymphoglandula ilio-coecalis.
6. Lymphoglandula coecalis.
7. Lymphoglandula mesentericae communis.

1. Lymphoglandula colicae descendents. This node is found close to the middle of the descending colon, imbedded in a small quantity of fat. It is somewhat round in shape, 2 mm. in diameter and 1 mm. thick.

Lymph from the descending colon drains into this node.

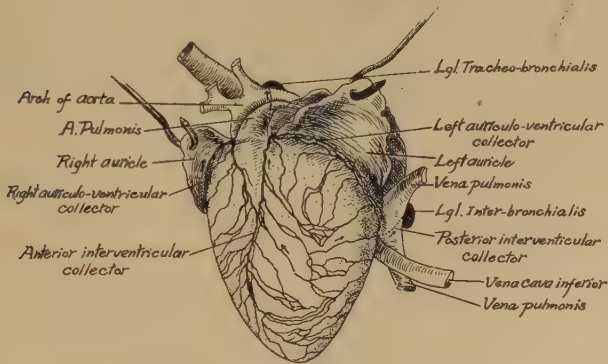


Fig. 8 Lymphatics of the heart

One small vessel from it follows the inferior mesenteric artery and at the origin of the latter joins with the lymph plexus surrounding the abdominal aorta.

2. The lymphoglandula colicae transversalis is located close to the middle of the transverse colon. It is round in shape, 5 mm. in diameter and 2 mm. in thickness.

The lymphatics of the transverse colon end in it and the efferent vessels usually reach the common mesenteric node.

3. Lymphoglandula colicae ascendents. This is found close to where the ascending colon joins the transverse colon. It is 4 mm. long, 3 mm. wide, 3 mm. thick and is not rarely accompanied by other very small glands close to it.

The majority of the afferent vessels draining into this node are from the ascending colon but a few vessels from the transverse colon also end here.

Lymph from it is received into the common mesenteric nodes.

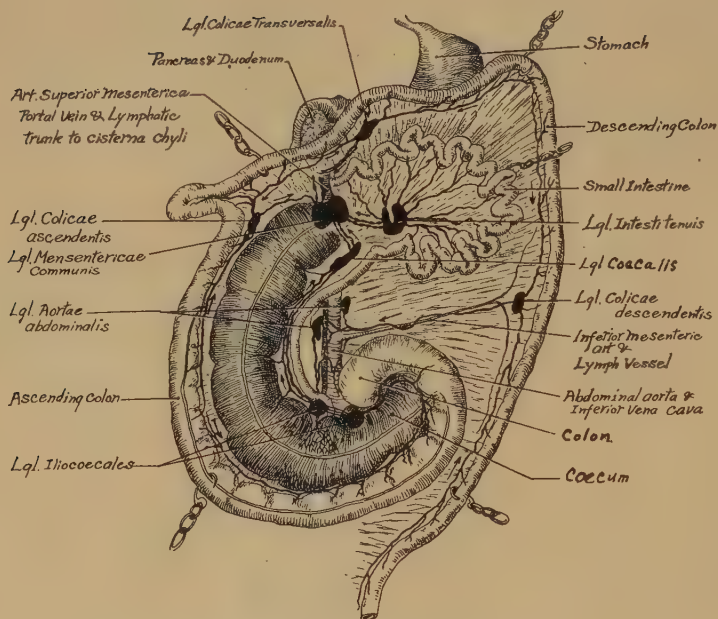


Fig. 9 Lymphatics of the intestine

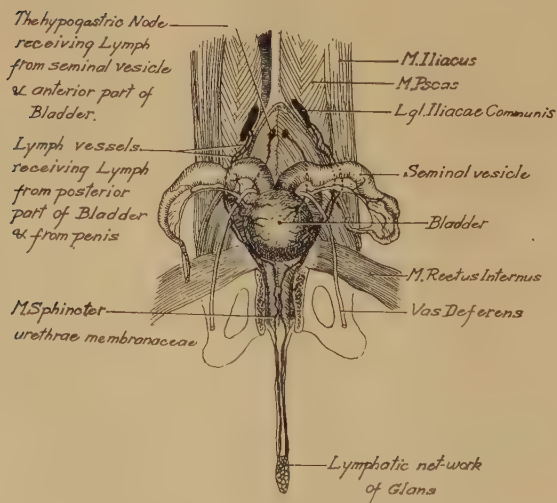


Fig. 10 Lymphatics of the genito-urinary organs

4. *Lymphoglandula intestini tenuis*. The gland of the small intestine lies near the center of the mesentery of the small intestine. It is an irregularly shaped node about 10 mm. in diameter and 2 to 3 mm. in thickness.

A large number of afferents from the small intestine converge toward it.

Two or three vessels from this gland terminate in the common mesenteric glands.

5. *Lymphoglandula ilio-coecalis*. A glandular mass constituting the ilio-coecal node is located around the spot where the ileum and caecum meet. This composite mass may be grouped into one node measuring about 10 mm. in diameter and 4 mm. in thickness but it is more often divided into two unequal masses.

The lymph vessels from a small portion of the ascending colon and from the caudal half of the caecum terminate in it.

Two or three vessels from this node traverse the mesentery stretched between the caecum and ileum to reach the coecal node.

6. *Lymphoglandula coecalis*. This node lies in the mesentery between the ileum and the caecum close to the common mesenteric node and the gland of the small intestine. Sometimes it fuses with the gland of the small intestine. It is somewhat elongated, measuring 7 to 10 mm. long, 3 mm. wide and 2 mm. thick.

Many of the coecal lymphatics terminate in this node, as do also the efferents of the ilio-coecal node.

Two or three large vessels from it end in the common mesenteric node.

7. *Lymphoglandula mesentericae communis*. This is the most important gland of the intestine. It lies on the trunks of the mesenteric blood vessels just before these cross the duodenum and is in close relation with the lymph gland which drains the small intestine and with the transverse colon and the head of the pancreas. It is often divided by the pancreas, one portion lying anterior and the other posterior to it. Behind this node lies the apex of the caecum and a portion of the duodenum. It is irregular in shape, about 8 mm. long and 3 mm. thick. In a number of cases it is not a separate node but forms one large mass with the glands of the small intestine and the appendix. However, separation into three distinct nodes is more common.

With the exception of the lymphatics from the descending colon, the entire lymph from the intestine drains into this node. Its afferent vessels come from the coecal, ilio-coecal and intestinal nodes.

The single large distinctly sacculated truncus lymphaticus intestinalis which leaves this node closely follows the course of the superior mesenteric artery and empties into the cisterna chyli. Often a small vessel is sent from this trunk to the retropancreatic node.

Lymphoglandula retropancreatica (fig. 11). This gland is found behind the head of the pancreas on the superior mesenteric vein near the foramen epiploicum. It is a pear-shaped or somewhat ovoid node about 8 mm. long.

A number of vessels from the liver and a small vessel from the common mesenteric node end in it.

One to four vessels are given off from this node and either join with the truncus intestinalis or follow the superior mesenteric artery and terminate in the cisterna chyli.

Lymphoglandulae aortae abdominalis (fig. 16) There are a large number of glands around the abdominal aorta and the inferior vena cava. These glands are divided into three groups: the inferior, middle and the superior. But it must be remarked that these divisions are absolutely artificial, for the nodes form a continuous chain without any distinct separation. Hence it is difficult to group separately. Some lymphatic vessels go to certain of these nodes but the territory drained is not distinct.

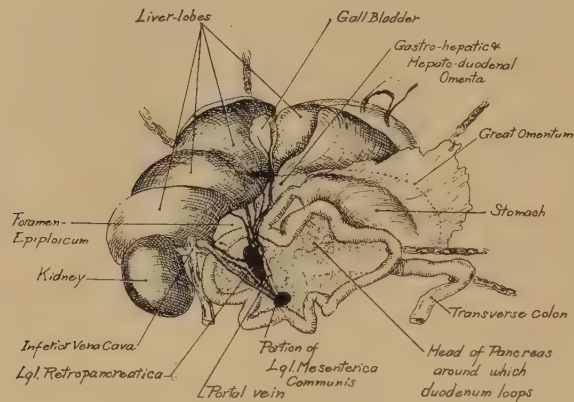


Fig. 11 Lymphatics of the liver

1. The lymphoglandulae aortae abdominalis inferiores are composed of three to six glands about the size of a pin's head which are grouped around the lower half of the great abdominal vessels, in meshes of the lymphatic plexus. These nodes are nothing more than 'Schalt-drüsen' interrupting the lymph from the hypogastric and the common iliac nodes and from the descending colon on its way to the cisterna chyli.

2. Lymphoglandulae aortae abdominalis mediales. This group of nodes is found around the abdominal aorta, the renal vessels and the superior mesenteric and spermatic arteries. The glands of this group are much larger than those of the inferior mesenteric arteries. They are usually ovoid in shape and the largest one may be as big as 5 mm. but very many small nodes may be found among them.

Since these glands lie in a plexus of lymphatic vessels, some of them are only interrupting nodes while others receive definite afferents from the kidneys and the testicles.

The efferent vessels from them terminate in the cisterna chyli which lies behind the aorta, between the pillars of the diaphragm.

3. *Lymphoglandula aortae abdominalis superior*. This node lies against the lateral surface of the pillars of the diaphragm. It is ovoid or round measuring about 4 mm. across.

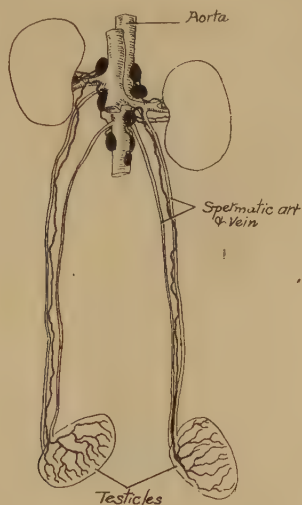


Fig. 12 Lymphatics of the testicle and kidney

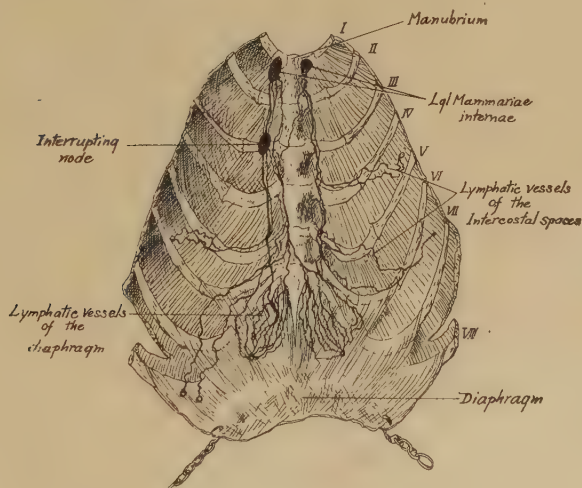


Fig. 13 Lymphatics of the diaphragm

Some afferents from the kidney and others from the abdominal plexus, which do not empty into the cisterna chyli at this level join it. Usually one of these vessels either penetrates the pillar of the diaphragm or passes behind the arcuate ligament and joins the cisterna chyli at once.

5. The lymph glands of the thorax

Lymphoglandulae arteriae mammae internae (figs. 13, 17). One node is located internal to the first rib in company with the internal mammary vessels. Cranially it is in relation with the origin of the sterno-hyoid and sterno-thyroid muscles and dorsally it is in relation with the innominate vein and the arch of the aorta. It is a small, somewhat round gland, measuring about 3 mm. in diameter.

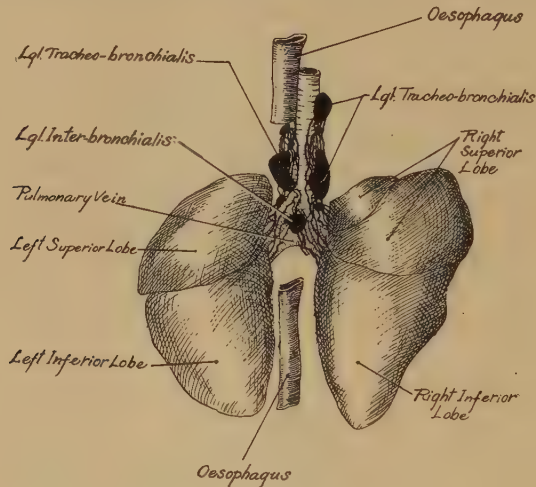


Fig. 14 Lymphatics of the lung

Lymphatics from the diaphragm and from the intercostal spaces terminate in this gland and it is usual to find that some vessels from the tracheo-bronchial nodes join it also.

The efferent vessels from the nodes of both sides form complicated plexuses on the innominate vein. The plexus on the left side joins with the ductus thoracicus and on the right with the cervical and the subclavian lymph trunks just before their termination in the vein.

Lymphoglandula inter-bronchialis (fig. 14). A single node is located at the bifurcation of the trachea between the roots of the bronchi. Ventrally it is in relation with the pulmonary vein and dorsally with the oesophagus. It is a somewhat spherical node measuring about 3 mm. in diameter.

The lymphatics of the lung and heart send some of their lymph vessels to it and the efferent vessels terminate in the tracheo-bronchial nodes of both sides.

Lymphoglandula tracheo-bronchiales (figs. 14, 17). These nodes lie in the angles between the trachea and bronchi on the right and left side respectively. They are elongated thick nodes 10 mm. long, 4 mm. wide and 3 mm. thick and are often separated only by the azygos vein which crosses the united nodes.

They receive afferents from the lung and the efferents from the bronchial node. The lymph vessels joining the glands of both sides of the trachea are often found to anastomose with each other and also with a number of anastomatic vessels which surround the trachea.

The efferent vessels from this node terminate in three possible places: in nodes along the internal mammary artery, in the lymphoglandulae axillaris prima costae or in the plexus in front of the innominate vein.

II. THE LYMPH VESSELS

6. *The lymphatic vessels of the head and neck*

1. *The lymphatics of the ear* (figs. 1, 15). The lymphatics of the ear are best injected on the medial side. The network appears near the margin and from this plexus appear a number of vessels which join with each other as they approach the root of the ear. The majority of these radicles pass to the parotid node but a few pass directly to the submaxillary lymph node.

2. *The lymphatics of the face* (figs. 1, 15). A large number of very fine vessels arise in the frontal region from the eye-lids, the side of the nose and from the upper lip. They all run toward the lateral surface of the neck, penetrate the platysma and come to lie between it and the masseter muscles. The larger collectors, however, follow the internal maxillary and branches of the external maxillary vein. All these vessels converge to the submaxillary lymph nodes. The lymphatics of the lower lip terminate in the inframandibular node.

3. *The cutaneous lymphatics of the neck* (fig. 15). All the cutaneous lymphatics of the neck which are subcutaneous at first penetrate the platysma. They then pass either through the dorsal or ventral border of the omotransversarius and terminate in the dorsal prescapular node.

4. *The lymphatics of the fore-limb* (figs. 3, 4, 15). When injections are made into the pad of the foot of the anterior limb, several vessels are seen to pass centrally in various directions. Some pass between the digits, others curve around the radial and ulnar borders of the foot to appear on the dorsum and still others pass to the flexor surface of the forearm. The latter have an entirely different path and termination from the former. Those that appear on the dorsum of the foot pass to the extensor surface of the forearm where they join to form one

vessel which accompanies the cephalic vein to the middle of the brachium. Here it leaves the vein and penetrates the panniculus carnosus, terminating at once in the retroscapular node.

Those vessels which appear on the flexor surface of the forearm continue their course as far as the distal third of the brachium where they meet the brachial artery. Here they join with the deep lymphatic vessels which accompany this artery and end in the axillary node.

5. *The lymphatics of the hind limb* (figs. 5, 6, 7, 15). A number of lymphatic vessels which arise from the medial and lateral margins of the foot arrange themselves on the lower part of the leg into medial and lateral collectors. The medial collector follows the saphenous vein very closely and terminates in the inguinal nodes. The lateral collectors pass along the outer surface of the leg and the majority terminate in the popliteal node. However, one or two vessels continue their course over the thigh and end in the abdomino-inguinal node. Indeed, the entire cutaneous lymphatics of the thigh converge to the latter nodes.

The deep lymphatics of the hind limb were not successfully injected except those along the femoral artery in the region of the thigh. If the pressure is relieved along the femoral artery by cutting away most of the adductor muscles, injections into the popliteal node always fill the deep lymphatic vessels which form a plexus around the artery and terminate in the deep inguinal nodes.

7. *The lymphatics of the abdomen*

1. *Cutaneous lymphatics of the abdomen* (fig. 15). Fine lymphatic vessels which arise from the skin of the abdominal wall soon penetrate the panniculus carnosus and join with one another as they converge to the abdomino-inguinal nodes. Separation of this area from that of the thorax is not sharply marked off for one merges into the other. Hence, an injection in the wide common zone which constitutes the boundary between them, may be seen to travel either to the external retroscapular or to the abdomino-inguinal node.

2. *The cutaneous lymphatics of the perineo-pudendal area* (fig 6). From the network of origin on the skin in the perineal and pudendal regions arise one or two collectors which pass between the thigh and the abdomen to end in the abdomino-inguinal node.

3. *The lymphatics of the bladder* (fig. 10). A fine lymphatic network from which a number of collectors leave is found on the bladder. These collectors are divided into anterior and posterior collecting trunks from the anterior and posterior surfaces of the bladder respectively. The anterior gain the brim of the pelvis and usually join the common iliac nodes while the posterior collectors run to the floor of the pelvis to reach the hypogastric node.

4. *The lymphatics of the seminal vesicles* (fig. 10). From the very fine network of origin on the surface of the seminal vesicle arise a number of collectors which join with each other at the free border of

the mesentery of the vesicle to form one common vessel which at the side of the bladder joins with the collectors from the posterior surface of the latter. This conjoined vessel runs over the floor of the pelvis to reach the hypogastric node.

5. *The lymphatics of the penis* (fig. 10). The lymphatics of the prepuce and glans form two collectors which run along the dorsum of the penis. They pass under the symphysis of the pubis, over the sphincter urethrae muscle. Close to the bladder they diverge and after reaching the pelvic brim follow the external iliac vessels and terminate in the common iliac nodes.

6. *The lymphatics of the testicle* (fig. 12). When injections are made into the substance of the testicle a large number of fine vessels appear on the surface. These vessels converge to the point where the spermatic vein leaves the testicle where they form common collectors. These collectors, two or three in number, follow the spermatic vessels. The artery and vein are closely interwoven by these lymphatic vessels in their entire course. Near the kidney, the lymphatic vessels leave the artery, but follow the vein and after reaching the wall of the renal vein terminate in some of the nodes of the middle abdomino-aortic group.

7. *The lymphatics of the kidney* (fig. 12). The deep lymphatics of the kidney are easily injected by simply forcing the injection mass into the kidney substance. A number of lymphatic vessels appear immediately at the hilum. These surround the renal blood vessels and terminate in the nodes in the middle abdominal aortic group.

8. *The lymphatics of the intestine* (fig. 9). Working out the lymphatics of the intestine is a difficult task in a small animal. Owing to the delicacy of the intestinal wall, direct injection with the needle was unsuccessful. In the caecum and a small portion of the large intestine, however, injections made on the teniae were quite successful in demonstrating the fine plexus on the walls. To inject the lymphatics of the other portion of the intestine, the following method was employed: (1) the intestine was cut into pieces about 8 cm. long, the mesentery being kept intact as much as possible. The contents were washed out thoroughly and a small quantity of rather coarse sand was introduced. This was rubbed against the intestinal wall by rolling the intestine between the fingers, so as to injure the interior of the intestine. The intestine was then flushed again and every drop of water pressed out. Into a piece of the intestine so treated the injection mass was then introduced quite fully and the ends clamped. By massaging gently with the fingers, the injection fluid was seen to slowly fill the lymphatics.

The lymphatics of the small intestine were also very well shown by the historical method. An animal which had fasted for a day, was fed with milk. After about one hour it was killed and the intestine examined. This method showed the collectors beautifully as fine white lines which could be traced to the cisterna chyli but their network of origin in the wall of the intestine was not recognizable.

The lymphatics of the intestine arise from a very closely meshed plexus in its wall. From this network several collectors run which pass through the glands placed in their course and finally form two efferents. One of them is the efferent of the node of the descending colon, which, following the inferior mesenteric artery contributes to the formation of the lymphatic plexus around the abdominal aorta. The other is the truncus intestinalis which connects the common mesenteric node with the cisterna chyli. This latter trunk carries the lymph stream from the entire intestine, except from the lower portion of the colon.

9. *The lymphatics of the liver* (fig. 11). The deep lymphatics are easily shown when injections are made into the hepatic tissue. A number of collectors appear at the hilum and pass together with the cystic duct and the superior mesenteric vein, between the two layers of the hepato-duodenal ligament in front of the epiploic foramen and reach the retropancreatic node.

10. *The lymphatic plexus around the abdominal aorta and inferior vena cava* (fig. 16). There is a very rich, closely-woven lymphatic plexus around the great abdominal vessels extending from the bifurcation of the abdominal aorta to the cisterna chyli. This plexus is further complicated by the presence of a number of glands, scattered in the meshes. These I classified as lymphoglandulae aortae abdominalis inferioris and medialis. The following are the contributors to the formation of this plexus: efferents from the common iliac and hypogastric nodes, those from the descending colon and the collecting vessels from the kidney and testicle.

This plexus is finest in its lower course and gradually forms itself into larger trunks as it continues upwards. Near the renal vessels, behind the aorta, between the pillars of the diaphragm it becomes a large cisterna chyli, into which the truncus intestinalis empties.

8. *The lymphatics of the thorax*

1. *The cutaneous lymphatics of the thorax* (fig. 15). The ventral lymphatics cover the pectoral area. The collectors which at first are subcutaneous penetrate the panniculus carnosus and lie between it and the pectoral muscles. They join with each other as they pass toward the axilla and converge to the axillary node.

The dorsal lymphatics cover the dorsal and lateral surfaces of the thorax and also the caudal portion of the scapular region. The collectors from this area, after penetrating the panniculus carnosus converge toward the retroscapular node.

2. *The lymphatics of the diaphragm* (fig. 13). The lymphatics of the diaphragm were injected only on the ventral surface of the muscular portion. A large number of the vessels are seen to join with one another on the diaphragm just behind the xiphoid cartilage whence they continue their course as the internal mammary trunks. If the injection is made on the diaphragm a little laterally, only a few vessels appear. These traverse a few lower intercostal spaces obliquely and likewise reach the internal mammary trunk.

3. *The lymphatics of the intercostal spaces* (fig. 13). The lymphatics of the intercostal spaces were injected successfully only in the anterior (ventral) portion. The vessel from each intercostal space has a slight cephalic obliquity. It traverses the intercostal space just beneath the parietal pleura and the transversus thoracis muscle and joins the internal mammary trunk almost at right angles.

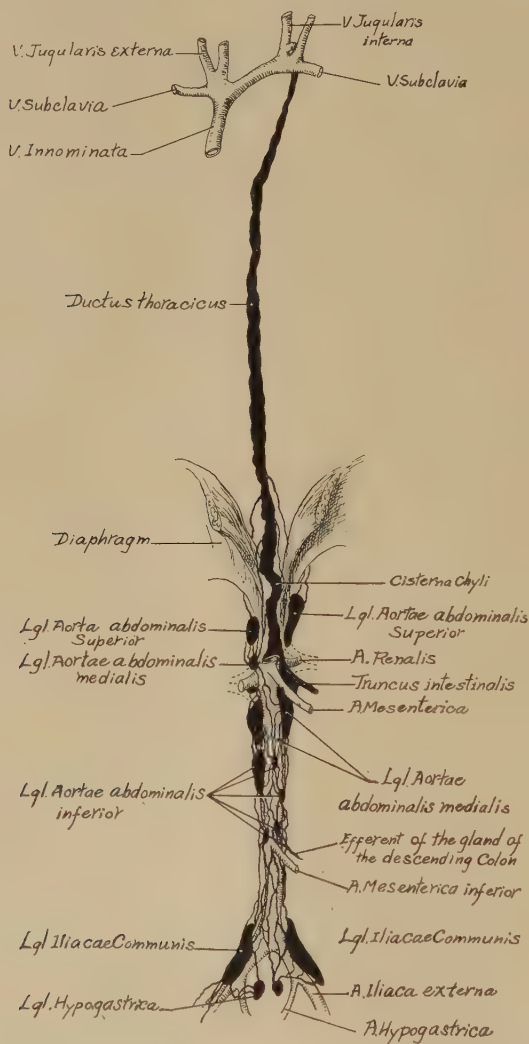


Fig. 16 The main central lymphatic nodes and thoracic duct

4. *The internal mammary trunk* (fig. 13). The internal mammary trunk lies directly lateral and internal to the sternum accompanying the internal mammary vessels. It is in relation ventrally with the intercostal muscles and dorsally with the transversus thoracis and the parietal pleura. It receives the lymph from the intercostal spaces and the diaphragm. In about 60 per cent of the cases, this trunk is interrupted by a small node placed a little above the middle of its course and behind the first rib. It opens into the internal mammary node.

5. *The lymphatics of the lung* (fig. 14). The deep lymphatics of the lung can easily be demonstrated, for by injecting deep into the lung tissue a number of vessels appear at the hilum. These vessels, at first,

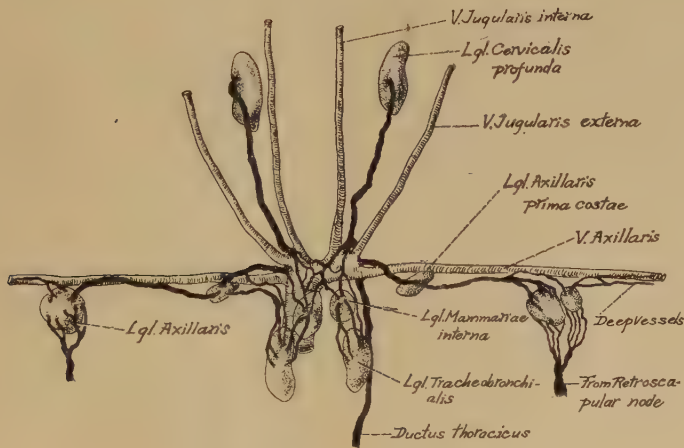


Fig. 17 The terminal vessels

surround the bronchi and then reach the tracheo-bronchial and bronchial nodes.

6. *The lymphatics of the heart* (fig. 8). The majority of the fine vessels which arise from the surface of the ventricle are directed toward the anterior and posterior interventricular groove and but comparatively few vessels pass to the auriculo-ventricular grooves. Thus there result in the heart, four main collectors: the anterior and posterior interventricular and the right and left auriculo-ventricular collectors.

The anterior interventricular collector follows the artery of the same name and a little above the middle of its course divides into two vessels which after receiving the auriculoventricular collector pass to opposite sides of the pulmonary artery, whence they run under the arch of the aorta, and terminate in tracheo-bronchial nodes on both sides of the trachea.



Figs. 18 and 19 Types of thoracic duct

The posterior interventricular collector runs in the interventricular grooves and passes over the bifurcation of the pulmonary vein to terminate in the inter-bronchial node.

7. *Ductus thoracicus* (figs. 16, 17). The thoracic duct arises from the cisterna chyli placed between the pillars of the diaphragm, behind the aorta. In the lower thorax, it lies in the plane dorsal to the aorta and ventral to quadratus lumborum and longus coli muscles but in the upper part it changes its course and runs obliquely to the left, lying behind the arch of the aorta and the subclavian artery. From here it passes directly cephalad to reach the root of the neck, where it bends downwards to terminate at the junction of the external and internal jugular veins.

The thoracic duct presented four different types (figs. 18, 19). It is a large single trunk distinctly sacculated, which lies for the most part, on the right side of the aorta. In the upper part of the thorax it crosses the longus coli muscle to reach the left side of the median line so as to lie dorsal to the arch of the aorta.

b. Two distinct vessels may constitute the thoracic duct, one on each side of the aorta; the one lies on the left side and passes vertically to the root of the neck while the other on the right side crosses the longus coli muscle and joins with the one on the left side just behind the arch of the aorta.

c. Two vessels are placed in exactly similar manner as in the preceding but they are connected by fine anastomatic vessels lying behind the aorta.

d. The thoracic duct may be represented by a continuous network dorsal to the aorta without a main trunk. This plexus, however, terminates in a single vessel exactly as the other types of duct.

I consider myself fortunate to have undertaken this investigation under Dr. Meyer's kind direction and am indebted to him for suggestions and assistance.

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EIN PAARIGER KNOCHEN AM UNTERRAND DER SQUAMA OCCIPITALIS

ADOLF H. SCHULTZ

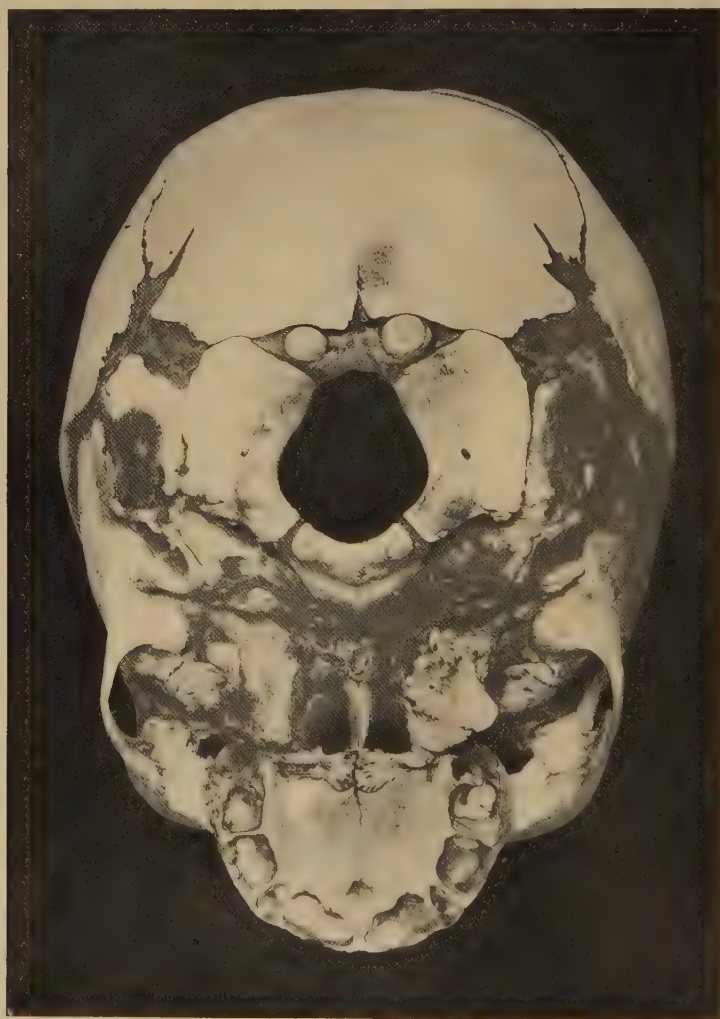
Carnegie Institution of Washington

MIT 2 FIGUREN IM TEXT

Die Entwicklung und die Anomalien der Squama occipitalis haben von jeher das Interesse der Autoren wachgerufen, wie dies aus der grossen Zahl der dieses Gebiet immer wieder von Neuem angreifenden Arbeiten hervorgeht. Selten decken sich die Resultate der verschiedenen Forscher, so dürfte denn diese Frage noch keineswegs als völlig gelöst betrachtet werden, wenn auch Aichel ('15) in neuester Zeit viel zu ihrer Klärung beigetragen hat. Von besonderem Interesse für die Entwicklung der Hinterhauptschuppe dürfte die Anomalie sein, die ich hier publiciere, da ich in der Literatur nur zwei ähnliche Fälle erwähnt fand (Weigner '12), dann aber auch, da die Anomalie die Unterschuppe betrifft und hier Abnormitäten seltener auftreten als an der Oberschuppe, ein Unterschied, der seine Begründung jedenfalls in der von Kölliker ('49) festgestellten, verschiedenen Entstehungsweise der beiden genannten Teile hat, denn während die Unterschuppe aus knorpliger Anlage hervorgeht, bildet sich die Oberschuppe aus Bindegewebsknochen.

Der Schädel eines neugeborenen Russen aus meiner Sammlung weist am Hinterrand des Foramen magnum zwei symmetrisch gelegene kleine Knochen auf, abgesehen von dieser Anomalie zeigt der Schädel ein durchaus normales Verhalten. Wie die beigegebene Figur 1 erkennen lässt, sind die Knöchelchen in knorpliges Bindegewebe gelagert, das sich zwischen den beiden Occipitalia lateralia und der Unterschuppe ausspannt. Dem Hinterrand der Partes laterales und dem Unterrand der Schuppe des Occipitale anliegend drängen die Knöchelchen die

ersteren vom Anschluss an die letztere ab, sodass die mediale Entfernung der beiden genannten Occipitalteile voneinander grösser ist, als dies in der Regel bei Neugeborenen der Fall zu sein pflegt. Der Rand der Unterschuppe ist an den Stellen, wo ihm die beiden überzähligen Knöchelchen anliegen, eingebuchtet. Die Knöchelchen selbst liegen 8 mm. voneinander entfernt, sind beide kreisrund und rechts 7 links 6 mm. im



FIGUR 1

Durchmesser. Während das linke Ossiculum die Gestalt einer abgeflachten Kugel zeigt, ist das rechte, grössere innen und aussen abgeplattet. Die Schuppe weist eine vom Unterrand ausgehende, kurze, mediane Spalte auf.

Für die Erklärung dieser allem Anschein nach äusserst seltenen Erscheinung lassen sich verschiedene Möglichkeiten anführen, aber nicht zuletzt dieser Umstand ist es, der mir, wie ich hier vorausschicken möchte, eine sichere Deutung unmöglich machte.

Unsere Knöchelchen sind dicker wie der Rand der Unterschuppe, aber ungefähr von derselben Dicke wie die Occipitalia lateralia, an deren Hinterrand sie sich anschmiegen, dies lässt es zunächst als nicht unmöglich erscheinen, dass ein genetischer Zusammenhang unserer Anomalie mit den Occipitalia lateralia besteht. Dieser Annahme wäre aber gegenüber zu halten, dass sich die letzteren stets aus je einem Stück entwickeln und das Neuauftreten eines zweiten Knochenkernes am hinteren Ende der Partes laterales des Occipitale ziemlich unwahrscheinlich ist.

Unter der Voraussetzung, dass unsere Knöchelchen der Unterschuppe des Occipitale zuzuzählen sind, lassen sich andere Erklärungen finden. Am unteren Rande der Schuppe ist nach den Untersuchungen von Lengnick ('97) vom vierten Monat des Embryonallebens an stets ein Knochen vorhanden, der später mit seiner Umgebung völlig verwächst. Es ist dieser Knochen zum ersten Mal von Kerekring (1670) beschrieben und später nach ihm als *Os Kerekringii* benannt worden. Die naheliegende Annahme, dass es sich in unserem Falle um ein paariges *Os Kerekringii* handle, scheint mir durch die Tatsache widerlegt zu sein, dass unter der grossen Zahl von als *Os Kerekringii* beschriebenen Fällen der Knochen stets unpaar auftrat, drei—oder viereckige Form aufwies und mit einer einzigen Ausnahme (Lucy '90, p. 21 " une fois, nous avons rencontré cet osselet complètement libre") mit der Unterschuppe in Verbindung stand. Selten ragt das *Os Kerekringii* über den Rand der Unterschuppe hinaus,—Fälle, die Virchow (57) zu der Bezeichnung *Manubrium squamae occipitalis* veranlassten,—weit

häufiger stellt es sich als ein durch zwei seitliche Rinnen begrenzter Teil am Unterrand der Schuppe dar, wie ich dies an einer Anzahl Schädel aus dem siebten, achten und neunten Monat beobachten konnte. Ein typisches Beispiel für letzteres Verhalten ist die beigegebene Abbildung 2 des Os Kerekringii an der Occipitalschuppe eines neugeborenen Negers.

Eine Erklärung, die, wenn vielleicht auch etwas gezwungen erscheinend, so doch nicht ganz von der Hand zu weisen ist, besteht in der Deutung unserer Knöchelchen als verlagerte



FIGUR 2

Ossificationscentren der Unterschuppe. Normalerweise entwickelt sich die letztere aus zwei Knochenkernen, die schon sehr früh miteinander verschmelzen. Mall ('06) beobachtete aber an jungen Embryonen eine vierteilige Unterschuppe, wobei die vier Teile nebeneinander gelagert sind. Es wäre nun nicht unmöglich, dass auch unserem Fall eine Unterschuppe mit vier Knochenkernen zu Grunde lag, wobei durch das intensivere Wachstum des einen Paares, das andere an die Basis der Schuppe gedrängt wurde und hier die beiden kleinen Knochen entstehen liess. Wieso sich diese hier in so compacter, regelmässiger Form ausbildeten ohne mit den benachbarten Teilen zu verwachsen, vermag ich nicht zu erklären.

*Zu der letzten Deutung, die ich hier geben möchte, regte mich die Untersuchung von Weigner an, der, wie anfangs erwähnt, zwei dem unsrigen ähnliche Fälle beobachtete. Die letzteren beiden sind folgendermassen beschrieben: pg. 161. "12. Schädel eines Neugeborenen. Der dorsale Rand des For. occip. m. besitzt einen keilartigen Ausläufer, im rechten, aussen abgestumpften dorsalen Knorpel befindet sich ein selbständiges Ossificationscentrum." Pg. 163. "20. Schädel eines einjährigen Kindes. Der dorsale Rand des For. occip. m. wird inmitten von den verschmolzenen dreieckigen Knorpeln gebildet, zu beiden Seiten treffen wir wie im Falle 12 Ossificationscentren." Weigner schliesst sich der hypothetischen Annahme Kollmanns von der Existenz des Occipitalwirbels an und setzt die an Feten- und Kinderschädeln zwischen den Occipitalia lateralia und der Squama occipitalis zu findenden Knorpelplatten dem mittleren knorpeligen Teil des dorsalen Atlasbogens analog. Diese Annahme würde unseren Knöchelchen, die ja in dem genannten Knorpel eingebettet liegen, eine Zusammengehörigkeit mit der Squama absprechen, um sie dafür dem Occipitalwirbel zuzuzählen, in dessen dorsalem Bogen sie auftreten, wo sie vielleicht den Epiphysen der in der Halsregion gabligen, am Atlas und Occipitalwirbel zurückgebildeten Processus spinosi analog zu stellen wären.

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Die übrige einschlägige Literatur ist vor Allem bei Aichel, Weigner und Lengnick zu finden.

COOPERATIVE TECHNIQUE

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A realization of the very great influence that technique has upon the character of the results obtained in microscopical studies doubtless has come home to every one with extended experience in this kind of work. Time serves only to strengthen the conviction that good methods of preparation are absolutely essential to worthy results. It would be going too far to say that this conviction is universal, otherwise we should have been spared much worthless and troublesome publication, but it can safely be said that there is more general appreciation of the refinements of microscopical methods each year. Up to the present most of our processes are the heritage from the pioneers in this field who gained them through purely empirical means. Comparatively little has been added within recent years. The time would seem to have come to undertake a serious study of the problems in this field with the hope of attaining a fuller knowledge of the processes upon which we are so dependent for all progress in our investigations. It is a most hopeful sign that many biologists are devoting careful attention to this phase of their work and that more precise results are now obtainable than formerly was the case. While much of this advance has resulted from mitochondrial studies, the whole subject of microscopic anatomy has benefited from the greater precision here demonstrated.

But when the individual investigator, thus convinced of the need for advance, faces the prospect of such an extended investigation as would be necessary to developing general principles of operation, applicable to a variety of organisms and tissues, he hesitates to undertake a course which would divert so much of his limited time to methods of preparation. The usual result is that he seeks out the most satisfactory means for han-

dling his own particular materials and contents himself with the best results obtained. Considering the welfare of the whole fraternity of microscopic anatomists this is a wasteful and time consuming method, because it requires, that, in large part, each investigator must work out his own technique and when he has done so his results to a considerable degree benefit only himself.

It would seem that there is a place here for the methods of combination and cooperation which have been so effective in business operations. That this thought is in the minds of not a few biologists is apparent from the inquiries that have come to me regarding the possibility of extending the investigations that are being carried on in the Zoology Department of the University of Pennsylvania to other objects than the ones serving there as objects of study. The increasing frequency of these has led me to consider the advisability of proposing a concerted series of studies upon technical processes, applicable to as wide a range of materials as our investigators have experience with, in the hope of developing more reasonable and more generally applicable preparation methods. In connection with such a series of studies there might be built up at some central place a collection of preparations, each the work of a specialist on some group, organ, tissue or cell, from which would be loaned for study and comparison, such slides as an investigator might need. In addition the results of these technical investigations might be published either separately or collected together and reduced to a more compact form. With the method and its results before him an investigator could determine whether he had attained success in his technique. It is probable that all microscopic anatomists are interested in this phase of their work and disposed to further it, but I do not know how many are convinced that advantage would accrue from a concerted series of studies or, if convinced, are disposed, or able, to cooperate. In the hope of determining whether it is possible to inaugurate correlated studies I am writing this note with the request that any who are interested send me their suggestions upon the subject. If the plan seems feasible I shall be glad to further it in any way possible.

A SIX-LEGGED RAT

SARA B. CONROW

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FIVE FIGURES

During February, 1915, a male albino rat (*Mus norvegicus albinus*) with six legs and a tail in a fixed position over its back, appeared in the rat colony of The Wistar Institute. Its ancestry was not known. Its external appearance while living may be seen in figures 1 and 2, and its appearance after death in figure 3.

Two short, deformed legs hung down loosely between the two normal hind legs. They dragged on the ground and were not under the control of the animal. The base of the tail was fixed firmly in an upward curve; the rest of the tail hung loosely on one side or the other of the body and also was not under the control of the animal. There were two sets of external genitals and a false anus at the left of and ventral to the functional anus. The animal was mated but never bred. It lived for about fifteen months in good health, when, seeming to be sick, it was killed on May 26, 1916, and examined. Its body weight was 249 grams, body length 210 mm., and its tail length about 192 mm.

The anterior part of this rat was normal as far caudad as the diaphragm, beyond which there were indications of doubling. The spleen was large. On the right side in normal position was a large kidney, while low down on the left side was the rudiment of a kidney (no kidney was found in normal position on the left side). The bladder was a double structure with a perforated, intermediate septum; there was an opening through each of the two penes. On the right side were one large testis, one very small testis, and one epididymis; on the left side also were one large testis, one very small testis, and one epididymis.

Skeletal conditions in the pelvic region may be seen in figures 4 and 5.

The vertebral column remained single throughout and was normal as far as the fifth lumbar vertebra. The fifth and sixth



Figs. 1 and 2 Caudal aspect, showing normal hind legs, rudimentary hind legs, scrota and curve of tail.

lumbar vertebrae were slightly deformed and twisted. The four sacral vertebrae were more deformed; the first had the left portion of the fused pelvic girdles attached to its left transverse process, while the second had the right portion attached to its right transverse process. The six proximal, caudal vertebrae were more or less deformed and were fixed firmly in a dorsal curve which turned to the left over the animal's back. The



3

Fig. 3 Ventral aspect; 1, penis, right side; 2, penis, left side; 3, papilla; 4, scrotum, right; 5, scrotum, left; 6, right rudimentary leg; 7, left rudimentary leg; 8, anus (position under); 9, false anus.

fused pelvic girdles and bones of the deformed legs will be described later.

The structures most concerned in the duplication in this rat were the pelvic girdle, hind legs, and genital organs. The two fused pelvic girdles showed three distinct parts, namely, a right nearly normal part, a left part 2 mm. shorter than the right and proportionally smaller, and a slender, irregular bar of bone 27.4 mm. long with ends flattened dorso-ventrally (figs. 4 and 5). These three were arranged as follows: The left part held a position

further forward than the right, the crest of its ilium being 7 mm. anterior to the crest of the right ilium. The right and left parts did not meet in the median line in the symphysis pubis but their posterior ends were separated laterally a distance of more than 2 cm. Bridging this space was the slender bar of bone firmly

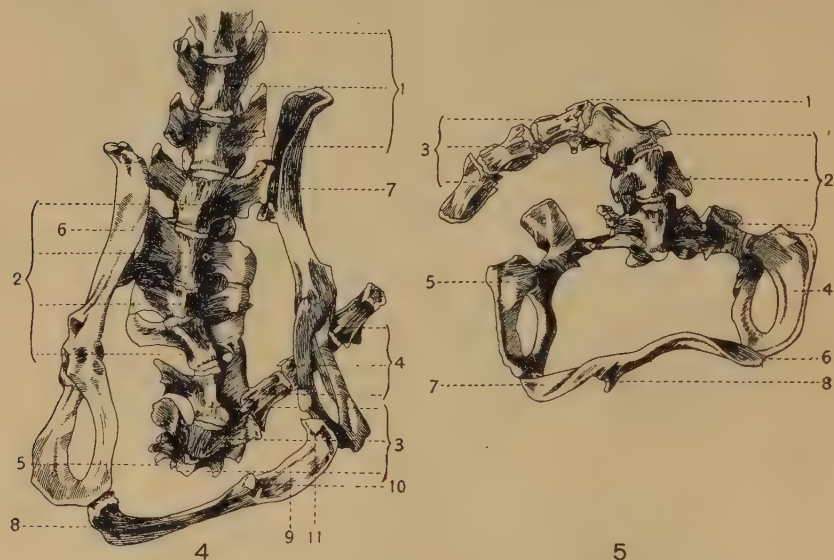


Fig. 4 Ventral aspect; 1, fourth, fifth and sixth lumbar vertebrae; 2, four sacral vertebrae; 3, first, second and third caudal vertebrae; 4, sixth, seventh and eighth caudal vertebrae; 5, curve of caudal vertebrae; 6, attachment of right part of fused pelvic girdles; 7, attachment of left part of fused pelvic girdles; 8, right deformed ilium; 9, left deformed ilium; 10, point of attachment of right rudimentary leg; 11, point of attachment of left rudimentary leg.

Fig. 5 Caudal aspect; 1, dorsal side; 2, third, fourth and fifth caudal vertebrae; 3, sixth, seventh and eighth caudal vertebrae; 4, right part of fused pelvic girdles; 5, left part of fused pelvic girdles; 6, right deformed ilium; 7, left deformed ilium; 8, point of attachment of right rudimentary leg.

attached by its flattened ends to the posterior parts of the right and left pubic bones. It seemed to be composed of two deformed fused ilia. From the ventral side of this bar hung the two short, deformed legs, one from its left end and one from a point at the left of the median line. They were attached by ligaments to slight pits in the bar at these points, and were scarcely more

than deformed feet, though each had two curved, slender bones suggesting the tibia and fibula. In addition to these bones each rudimentary leg had a deformed os calcis and the rudiment of another bone of the tarsus; in the right leg there were four metatarsal bones and four digits, all more or less deformed; in the left leg there were three metatarsal bones and three digits, less deformed than in the right, but not quite normal.

The two sets of external genitals were normally placed with reference to the two pairs of legs; namely, one set for the right normal hind leg and the right deformed leg, and the other set for the left deformed leg and the left normal hind leg. The two penes were 2.5 cm. apart and there was a fleshy papilla about midway between them. Internal conditions here have been noted.

Looking at the double portion of this animal it is seen that the right component is more fully developed than the left and that it is nearly in normal position, while the left component is wholly at the left of the median line. The two parts of the right pelvic girdle are larger than those of the left component, the right part being nearly normal and the left part being the larger of the two fused ilia and having a more pronounced acetabulum. The left leg of the right component (the right deformed leg) has more indications of tibia and fibula and more bones in the foot than has the other deformed leg. Also the functioning anus is associated with parts of the right component.

This rat apparently corresponds to the dipygus type of human monster, although its vertebral column remains single throughout. Examples of this type have been described by Gould and Pyle ('97) and by Wilder ('04). In such monsters the pelvis and the lower part of the spinal column are more or less double. Wilder ('04) describes several human monsters of this type. He gives first Wells' case of Mrs. B. Here the duplication began at the third lumbar vertebra. There were a fused double pelvis, two pairs of legs (the inner ones somewhat reduced in size), two sets of external genitals, two bladders, two ani, and two rectums. Heschel's case was of a girl seventeen years old with double parts below the second lumbar vertebra. In the case of

Blanche Dumas the pelvis appeared incompletely double and there were two extra legs, two distinct sets of genitals, and a rudimentary mamma just above the pubes. Others described by Wilder ('04) which were not strictly of the dipygus-type were as follows: Jean Baptista dos Santos was wholly normal save in the pelvic region. Here there was an exact duplication of the external genitals and a median third leg, which was double and symmetrical, depended from an extra, median pelvic bone. There were four testes present. (Gould and Pyle ('97) in describing this case say that here there was possibly a double bladder communicating by an imperfect septum.) Bechlinger's case was that of a female counterpart of dos Santos. The genitals were duplicated, a third leg was attached to a continuation of the processus coccygeus of the sacrum, and there were two rudimentary mammae close together above the pubes. In the case of Louise L. there were two atrophied legs on a rudimentary pelvis attached ventrally to the normal pelvis, with two rudimentary mammae at the insertion of the legs. There was no duplication of the genital organs.

Gould and Pyle ('97), besides describing these same monsters, give also examples of differing degrees of duplication in the posterior part of both males and females, but the cases already cited correspond most nearly to conditions found in the male albino rat which has been described.

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THE FIXATION OF MAMMALIAN CHROMOSOMES

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TWO TEXT FIGURES AND THREE PLATES

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INTRODUCTION

The advance of mammalian cytology has been exceedingly slow, due partly to the difficulty of obtaining material showing the proper stages of mitosis in sufficient numbers but largely to the fact that the various methods of technique in use were not at all successful in preserving the structures of these small cells. The first difficulty is obviated by securing material from a large number of animals and examining a small portion of tissue from each animal (not more than one or two slides). Eventually a tissue may be found that is in, what might be called, a 'cycle of division,' when the mitotic figures are present in large numbers and a choice of good stages is readily made. This is true for germ as well as somatic tissue. The question of the preparation of mammalian tissue for cytological work forms the body of this paper.

During the early part of this work I had the benefit of the experience of Dr. Ezra Allen in his studies on the fixation of rat tissues (1) and Dr. McClung has assisted me all through my

experiments with suggestions and criticisms. To these men I am exceedingly grateful.

Several years ago I began a study of the chromosomes of the pig but this had to be abandoned because of the hopelessness of analyzing the chromosome complexes. Even as my figures were then, they were as good as most of the published figures of mammalian mitosis. Since unlimited material was needed for experiments on technique and since there was a fairly large supply of cats available, the early work was carried out on cat testes and the method most successful on this tissue was tried on various other mammals. For some reason I have been unable to obtain the beautiful cytological preparations that Dr. Allen gets by the use of his picro-formol-acetic-chromic mixture, although I get excellent general fixation with it. This may possibly be due to the fact that his fixing fluid was developed with special reference to the rat. With the following method, however, I get very constant results which are, I believe, comparable in every respect to those obtained by Dr. Allen. Furthermore the fixation is not only constant for one mammal but has worked with uniformly good results on seven mammals and on the somatic as well as the germ cells. With two available methods in the field the difficulties besetting mammalian cytology should be much reduced.

THE FIXATIVES EXPERIMENTED WITH

In the experiments on technique that have been carried on in the Zoological Laboratory of the University of Pennsylvania for a number of years it has been found of value to vary the temperature at which fixing fluids are used. All the fluids tried by me were used as indicated at blood heat and at about 4 or 5°C. As I had previously failed to obtain good results at room temperature I did not, except in a few instances, experiment further at this degree.

An attempt was also made to study the effect of varying the time between the removal of the tissue from the body and the placing of it in the fixing medium. In the following table the

fluids used are outlined and also the methods of applying each fixative. In many cases the experiment was repeated when there seemed any chance of obtaining better results with that particular agent.

Table of fixatives

FLUID	TEMPERATURE	TIME IN FLUID	TIME THAT ELAPSED BEFORE FIXATION
	<i>deg. C.</i>	<i>hours</i>	
Bouin.....	38 and 4	24	Immediate
B.2 + urea (1)	38 and 4	21, 23, 24 and 49	Immediate and in 15 minutes
B.3 (2).....	38	24	Immediate
B.3 + urea.....	38 and 4	24	Immediate
Carnoy.....	4	24 and 49	Immediate
Ohlmacher.....	4	24 and 48	Immediate and in 15 minutes
King's solution.....	4	4, 6, 21 and 25	Immediate
Allen's solution No. 15	38	24	Immediate
No. 16.....	38	24	Immediate
Flemming strong solution.....	Room, 38 and 4	24, 28, 30 and 49	Immediate
Flemming + urea strong solution	38 and 4	24 and 49	Immediate, 10, 15, 20, 25, 30, 45, 60 minutes and 4½ hours.

(1) and (2). B.2 and B.3 are the symbols which are used in this Laboratory to designate two modifications of the picro-formol-acetic preservative. These modifications are:

<i>Saturated</i>	<i>B.2 parts</i>	<i>B.3 parts</i>
Picric acid, aqueous solution.....	75	75
Formalin.....	10	15
Glacial acetic acid.....	10	10

To these two solutions are sometimes added 0.5 gram of urea per hundred cubic centimeters.

In controlling the temperature of the fluids I have made use of our constant temperature rooms in the case of material fixed at 38°C. When the fluids were used cold the vials containing the fixatives were packed in ice in a quart 'Thermos' vacuum jar having a very wide mouth. As this jar is provided with a handle

it is very convenient to carry about when it is necessary to go some distance for material. Flemming's solution when kept on ice registers about 4° to 5°C. Ice can be kept in this jar for several days.

The results of the experiments

The judgment that is passed on material prepared for cytological work must be based on two points:

1. *The general fixation.* If this is not good (i.e., if shrinkage or general distortion is evident) it paves the way for a very just criticism of any conclusions that may be drawn from the study of the chromosomes in such a tissue. As a rule if the general fixation is poor the chromosomes are not very likely to be decipherable, but should they appear so in shrunken and poorly fixed tissue they might well be regarded as probably abnormal.

2. *The clearness* of the various stages of the chromosomes and particularly, I think, of the *distinctness*, *differentiation* and *separation* of the chromosomes in the metaphase plate. In mammals, chromosomes that are more or less lumped together, even when it is apparently possible to distinguish the separate elements, do not give a true picture, in either form or number, of the actual conditions. This, I believe, I will be able to demonstrate a little later in this paper. Accepting material in such condition as workable has led several investigators of mammalian chromosomes rather far astray.

With these points in mind the material fixed by the above methods was studied. Most of the fluids used gave good preparations for general histological structure but the chromosomes were clumped and indistinguishable one from another. The first result obtained that approached the fulfillment of the required conditions was when cat testes was placed in cold (4° to 5°C.) Flemming's solution to which had been added a little urea. Previous to placing it in the fluid the tissue had been allowed to remain on a glass plate for approximately from ten to twenty minutes. Urea was added, as it had been found in the work of other members of the Laboratory to increase penetration. The

amount taken has never been accurately determined but a few crystals are added to 5 or 10 cc. of fluid bringing the concentration of the urea in the fixative probably to about 0.5 per cent. It is difficult even to surmise why this method of delayed fixation should prove successful. It might be suggested that evaporation or exosmosis might play a part in separating the chromosomes although if this were true we would expect to find considerable shrinkage of the tissue. Slight shrinkage is to be observed in tissue treated in this manner although the chromosomes appear to be in excellent condition as is evidenced by figures 3, 5, 6, 7, 8, 9 and 11. This procedure gave excellent separation of the chromosomes in the testes and ovaries from a number of cats and with the testes of a few pigs. It was successful with the only cat embryo that was fixed in this way.

It was soon found that mammalian tissue could be fixed with better results, as far as lack of shrinkage was concerned, than those obtained by the above method if the material was cut into very small pieces (not more than 2 or 3 mm. in diameter) and placed immediately into cold Flemming plus urea. It is well not only to cut small pieces but to actually tease the tissue finely in the cold Flemming solution so as to obtain rapid penetration. When pieces too large are placed in the fixing solution good results are only obtained in a narrow area around the outside of the piece. The tissue may or may not blacken when placed in the cold Flemming. The absence of the usual blackness is not necessarily indicative of poor fixation.

WASHING AND DEHYDRATION

All Flemming fixed material was washed for about twenty-four hours in running water or, when this was impractical, many changes of water were made. Dr. Allen's automatic dehydrating device (1) is a great saver of time as it does away with the necessity of handling the material so many times. In the Laboratory I use it constantly but it is impossible to make use of it in field work. I have obtained as good results by dehydrating in the usual way making the steps in the ascending series of alcohols very gradual.

CLEARING AND EMBEDDING

When the material is in 95 per cent alcohol cedar oil is added and is usually allowed to remain on the tissue at least twelve hours, one or two changes being made during this time. The cedar oil is displaced with xylol and the material is embedded in paraffin in the usual way (2).

DISCUSSION OF THE SUCCESSFUL METHODS OF FIXATION

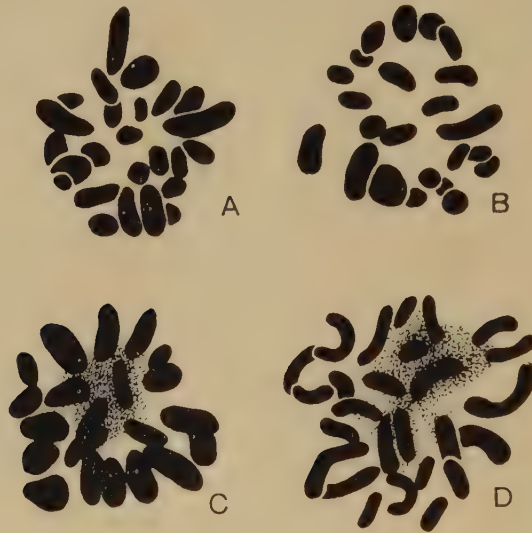
The latter method (i.e., involving the immediate placing of the tissue in the fluid) is not, as is the first, open to the possible criticism that the length of time between the removal of the tissue from the body and the placing of it in the fixing medium might allow pathological changes to enter. At present I am not of the opinion that the short wait between the removal and killing does produce anything abnormal other than a slight shrinkage. The chromosomes appear sharp and well separated and exhibit considerable differentiation. Some difference in the general morphology of the chromosomes is apparent in figures 10 and 11. Figure 10 is from the testes of a guinea-pig that was fixed immediately while figure 11 is from a testis of another individual fixed by the 'delayed' method ten minutes). The chromosomes in the 'delayed' fixation (fig. 11) are thinner and less differentiated than those of figure 10 although they are better separated. Whether this is a result of the method of preparation or not I cannot say at present. It will take considerable study and comparison to determine exactly whether this technique produces any abnormalities in the chromosomes and this study I have not completed as yet. However, since the plan of immediate fixation in cold Flemming plus urea has proved universally successful with mammalian tissue in my work, and as it is not open to the criticism pointed out above, I should suggest that it be followed in preference to the other method, using the delayed fixation only in case of the possible failure of immediate fixation.

Another point of great importance—no matter which method is followed—is that the material used must be absolutely fresh. This cannot be emphasized too strongly and is another condition

that may help to account for some of the inaccuracies in the published results of mammalian chromosome studies. Early in my work on cat testes I found that testes removed from cats that had been dead not more than a half hour and whose bodies were still warm were hopeless for a chromosome study. Recently I have confirmed this observation on the testes of ten pigs which were received after having been removed from the animals for from ten to thirty minutes. The general fixation was excellent but the chromosomes were clumped together¹ and there was no recognizable differentiation in the size and shape of the chromosomes such as is discernible in tissue which has been killed when absolutely fresh. In text-figure 1 are shown the round shapeless masses of chromatin that result from improper fixation or from the fixation of stale tissue. Such figures do not give a true picture of the actual structure of the chromosomes and it seems very likely that frequently several chromosomes may be fused thus giving an erroneous impression of the total number. I recently prepared the testes, as they were removed, from eight pigs and the fixation (immediate immersion in cold Flemming) was excellent in each case. Figure 13 was drawn from a cell in this lot of material. Text figure 2 is a photograph of a brain cell in a well fixed pig embryo. Judging from the number of chromosomes that have been reported for the pig (eighteen) (3), my own early results (about twenty-four) on this animal in comparison with my present results (over forty) it would seem exceedingly probable that a fusion had taken place between many of the chromosomes in the studies showing lower numbers. This cannot be accounted for by assuming that a difference in the breed of pig might cause the great variation as I have, among others, material from the same breed as reported on by Wodse-dalek (Poland China). Figure 3 represents a spermatogonial division in a Poland China boar. It might be suggested at this

¹ The tendency for closely associated chromosomes to fuse and appear as one when poorly fixed has been strikingly illustrated in the studies of Dr. P. W. Whiting and myself on the chromosomes of the mosquito. In these studies the actual conditions, particularly in the somatic cells, were long obscured by the tendency for closely approximated chromosomes to run together. This condition has been discussed in our papers (4 and 3).

point that staleness of tissue as well as improper technique may account for the great variation in the number of chromosomes reported for human material as it is very difficult to obtain this tissue in an absolutely fresh condition. It might also be suggested in view of the fact that all the mammals studied by me have over forty chromosomes that when a perfect fixation of human material is attained the number of chromosomes will be



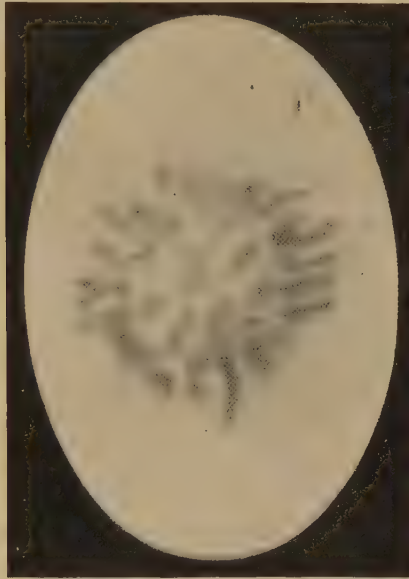
Text fig. 1 All four figures represent spermatogonia of the pig in metaphase.

A and *B* illustrate the effect of poor fixation on fresh tissue. The unequal size of the chromosomes would seem to indicate that a fusion between various elements has taken place, and the massing together of these bodies in many cases make it impossible to distinguish the line of separation. Note that the chromosomes tend to be globular in form and lack differentiation.

C and *D* show the effect of the new method of fixation (immediate immersion in killing fluid) on stale tissue. *C* was taken from a testicle that had probably been removed from the animal for a longer period of time than had *D*. The large size and small number of the chromosomes of *C* again suggest that a fusion between chromosomes has taken place. The stippled area is dense and very indistinct. *D* shows a cell in a slightly better degree of preservation. The chromosomes are more numerous and better differentiated than in *C*. The form compares more favorably with the morphology of the chromosomes found in properly preserved material. In *D* there is also considerable density and lack of distinctness in the center of the chromosome plate indicated in the drawing by the stippled portion. Compare these drawings with figures 1, 2, 3, 4 and 13.

nearer that reported by Winiwarter (forty-eight) than that reported by other investigators.

The fact that a testis that remains in a dead body for a half hour or stands in a gross condition after removal from the body for a similar length of time or even less, shows the chromosomes clumped while tissue that is freshly removed, cut into small pieces and allowed to stand for ten to twenty minutes before



Text fig. 2. This is an enlarged microphotograph of the metaphase chromosomes from the brain of a pig embryo. Figure 2 is a drawing of this cell.

immersion in the killing fluid presents the mitotic figures in apparently excellent condition seems to be contradictory. For the present the fact must stand without explanation as I can offer no suggestion as to the possible reason for this behavior.

It seems probable that the explanation for the success of the cold fixation may lie in the suppression of metabolic activities when the tissue is immersed in the cold medium and the preservation of the living structures until the fluid is able to penetrate and fix them permanently.

A notable feature of the immediate fixation of mammalian tissue in cold Flemming is the perfect preservation of the extremely delicate splits between chromosomes and of the chromosome forms found in the spermatocyte divisions. These figures have seldom been demonstrated in mammalian studies. Synizesis was not seen in this material except in the center of a piece of tissue which was rather large and where the fixative had not penetrated. Even here it is not the tight ball of threads figured by so many but gives every evidence that it is the result of the extraction of fluid. In my preparations where any evidence of the synizesis of the thin chromatin threads occurs, there is only a slight contraction of the threads away from the nuclear wall appearing as though the fluid which had supported these threads had been removed. The shrinkage usually appears to be equal from all sides although occasionally a cell is found with the chromatin threads massed at one side. In well teased or small pieces of tissue these same stages appear with the threads well separated and there is no shrinkage away from the nuclear wall. That there is something different at this stage is evident but the difference appears to me to be purely a physical one.

I have been much interested in the universality in application of the two methods of fixation of mammalian chromosomes outlined above, I have obtained excellent results with them on the following tissues in the number of cases indicated under the tissue in question. The number in parenthesis under the column

	TESTES	OVARY	EMBRYO	METHOD OF FIXATION
Cat.....	13	7	1	Delayed (5, 6, 7) and immediate (17)
Rat.....	4			Delayed and immediate (14 and 15)
Guinea-pig.....	2			Delayed (11) and immediate (10)
Pig.....	13		Several	Delayed (3) and immediate (1, 2, 4 and 13)
Rabbit.....	1			Delayed (8, 9)
Bat.....		2		Immediate (12)
Mouse.....	4			Immediate (16 and 18)

headed "Method of Fixation" refer to the figures illustrating the conditions found in the material. As used above 'delayed' indicates that the tissue has been allowed to stand in open after having been removed from the body (for from ten to twenty minutes) while 'immediate' indicates that the tissue had been cut into very fine pieces or teased and plunged immediately into the cold Flemming solution upon removal from the body.

In the case of the pig most of my material has been preserved by the immediate immersion in cold Flemming's solution plus urea. I have a large number of embryos fixed in this manner and as yet have examined only a few. Each one studied has shown uniformly good fixation so far and it may reasonably be expected that the remainder will be equally good since it is fairly evident that the results are not due to a happy accident in one or two isolated cases.

THE METHOD IN BRIEF

To fix mammalian material for cytological study:

1. Obtain fresh specimens from as many different animals as possible so as to be sure of obtaining one or more in a 'cycle of division.'

2. Place small or finely teased pieces of fresh tissue immediately into cold Flemming's solution plus urea. Allow to remain twenty to twenty-four or more hours.

3. It is suggested that this second method be not used except in case of the failure of the preceding one. Allow small pieces of fresh tissue to remain in the air for from ten to twenty minutes after removal from the animal before placing them in Flemming's solution plus a little urea which is used at a temperature of about 4°C. Allow the material to remain in the fluid for twenty to twenty-four or more hours.

4. Wash in water for about twenty-four hours.

5. Dehydrate by very gradual steps.

6. Clear from 95 per cent alcohol in cedar oil followed by xylol.

7. Imbed in paraffin.

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EXPLANATION OF PLATES

I have used drawings instead of photographs to illustrate the state of preservation in the various cells since drawings show the chromosomes much more satisfactorily than do photographs in most instances. Furthermore it is difficult to find cells in animals possessing so many chromosomes in which the metaphase plate is flat enough to photograph well although it may be perfectly easy to draw the same plate. I have included one photograph, which, though not good, is probably sufficiently clear to give an idea of the separation of the chromosomes and of their large number (text-fig. 2). Figure 2 is a drawing of the same cell.

All figures were drawn using a Zeiss apochromatic, 1.5 mm. objective and the compensating ocular 12 $\times$ which gives an original magnification of 3400 $\times$. These drawings were enlarged to twice the original magnification and reduced to approximately 4500 $\times$ in reproduction.

PLATE 1

EXPLANATION OF FIGURES

- 1 Prophase (late) from the amnion of a pig embryo. Immediate fixation.
- 2 Polar view of metaphase from the brain of a pig embryo. Immediate fixation.
- 3 Polar view of metaphase of spermatogonium of pig. Delayed fixation.
- 4 Polar view of metaphase from the brain of a pig embryo. Immediate fixation.
- 5 and 6 Polar views of metaphase of spermatogonia of cat. Delayed fixation.

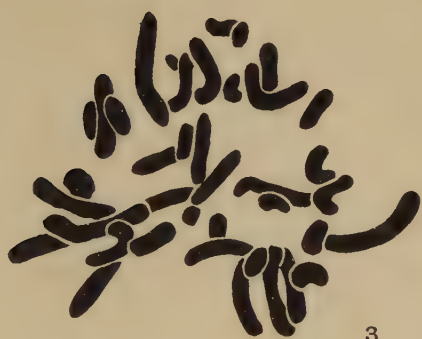


PLATE 2

EXPLANATION OF FIGURES

7 a and b Polar view of spermatogonium of a cat found in two sections. Delayed fixation.

8 Side view of the metaphase plate in the spermatogonium of a rabbit. Delayed fixation.

9 Prophase of spermatogonium of rabbit. Delayed fixation.

10 Polar view of metaphase of spermatogonium of a guinea-pig. Immediate fixation.

11 Polar view of metaphase of the spermatogonium of a guinea pig. Delayed fixation. Note the difference in the chromosomes figures 10 and 11.

12 Polar view of metaphase in the ovary of a bat. Immediate fixation.

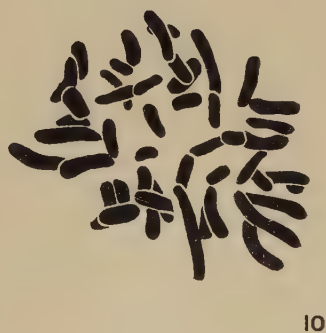
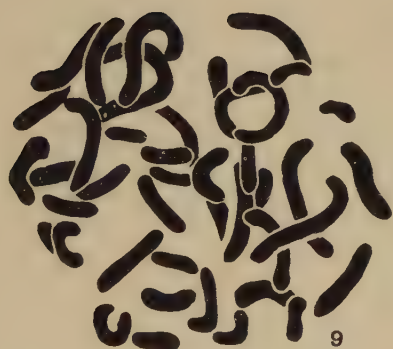
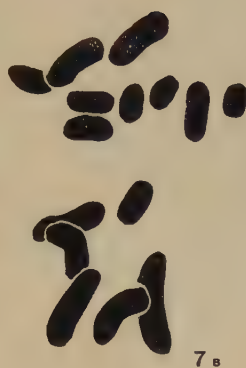
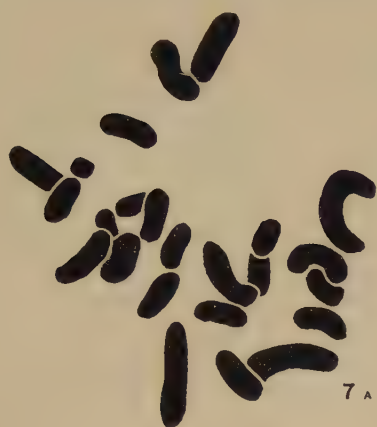


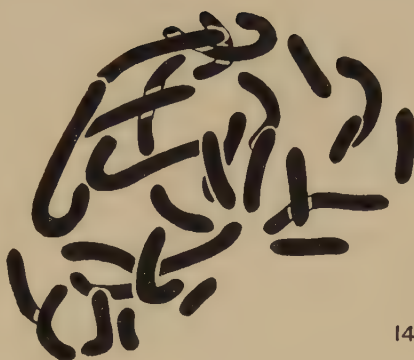
PLATE 3

EXPLANATION OF FIGURES

- 13 Polar view of metaphase of spermatogonium of pig. Immediate fixation.
- 14 Late prophase of spermatogonium of rat. Immediate fixation. This is probably a cut cell.
- 15 Metaphase of spermatogonium of rat. Immediate fixation. This is a cut cell.
- 16 Early prophase figures from a first spermatocyte of a mouse. This is only a portion of the cell. Immediate fixation.
- 17 First spermatocyte figures from the testes of a cat. Note the characteristic forms that have been reported for other forms but seldom for mammals. Immediate fixation. Such forms have been found in all the mammalian tissue studied.
- 18 A first spermatocyte from the testes of a mouse. An oblique view. Immediate fixation. This is probably a cut cell.



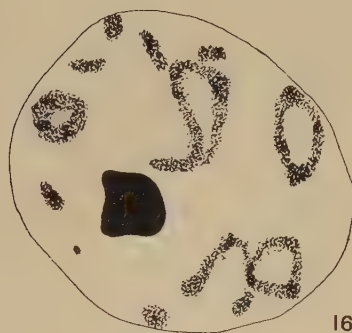
13



14



15



16



17



18

HERMAPHRODITISM IN FUNDULUS HETEROCLITUS

F. E. CHIDESTER

From the Zoological Laboratory, Rutgers College, and the Marine Biological Laboratory, Woods Hole, Mass.

THREE FIGURES

The scanty literature on hermaphroditism in fishes contains no reference to a case of simultaneous maturation of spermatozoa and ova in any species of *Fundulus*. In fact relatively few cases of hermaphroditism have been recorded in the teleosts, the only other case in *Fundulus* being in *Fundulus majalis* (Newman, '08).

The specimen to be described is interesting because it is the first known case of hermaphroditism in a species much used in experimental embryology; because it was a conspicuously well marked male in external appearance; and because the gonads which gave the secondary sex characters were entirely male and in no way attached to the egg masses. Lastly the diffuse egg masses were found in close association with the organs of digestion and in some cases occupying positions under the serous coats of the organs.

In June, 1915, while occupying a research room at Woods Hole through the courtesy of Dr. F. R. Lillie, Director of the Marine Biological Laboratory, it was desired to fertilize the eggs of a female *Fundulus heteroclitus* by the sperm of a vigorous male. A brightly colored active male measuring 7.5 cm. in length was selected from the aquarium and lightly tested for milt. The milt exuded readily and the individual was conveyed to the experimental desk and placed conveniently near in a finger bowl about one-third full of sea water, another finger bowl being laid partly over the first to prevent an escape. A few moments later, the supposed male was stripped over the selected eggs. A half dozen apparently normal transparent eggs issued from the short vas deferens. The animal was again made captive in the finger

bowl and the eggs placed therewith in the hope that sperms adherent to the body from the trial stripping might still be capable of fertilization.

A half hour later the specimen was again stripped lightly and three more eggs, a total of nine, were secured. The eggs which had previously been placed with their fraternal sperm and had been in a rather large quantity of water were examined and showed no signs of development. Four of the nine eggs (the three last secured and one of the others) were placed in a finger bowl with a very little sea water on the bottom, milt from a normal male was added to them and to a new lot of eggs from a normal female. Three hours later it was found that most of the control eggs had reached the 8 cell stage but that the eggs from the hermaphrodite had failed to develop with either lot of sperms. Later examination showed no initiation of development.

Since my stay at Woods Hole was short and it was impossible to keep the specimen under observation, it was quickly killed in Kleinenberg, then removed and opened for the penetration of the fluid. Before replacing the opened animal in the killing fluid, small portions of the testes were removed and placed under the microscope for comparison with normal testes. The sperms were apparently the same in structure and activity as normal sperms examined at the same time. Fresh testis macerated and placed with normal eggs failed, however, to initiate development.

Careful examination of the hermaphrodite was made and it was compared externally with normal males and females.

The male *Fundulus heteroclitus* reaches a length of about 5 inches and is easily distinguishable at all ages and in all seasons by the presence of a number of transversely arranged silver bars on the sides and usually by a yellowish or orange colored belly. The ground work of the body is dark green and in mature specimens at the breeding season there are numerous white and pale yellow spots of color on the sides. The dorsal fin of the male bears a dark spot at the base of the last rays; in the young male this is subdivided into two blotches. The vas deferens extends to the anal fin or even a little way along the anterior ray. The dor-

sal and anal fins bear small papillae termed by Newman ('09) contact organs, which are used in holding more firmly to the female when mating.

The young female has dark bands like the silver bands of the male and during the spawning season some older females show the dark bands against their olive ground color. The oviduct

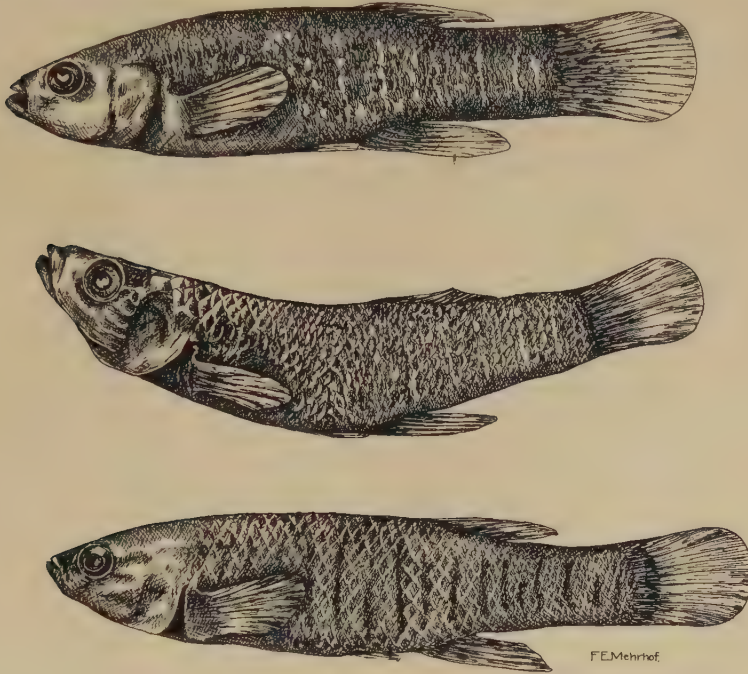


Fig. 1 A normal male, the hermaphrodite and a normal female of the species *Fundulus heteroclitus*. Drawn from a photograph by Mr. J. G. Hubbard with details from the specimens.

extends along the anterior ray of the anal fin about two-thirds its distance.

The hermaphrodite was marked like a male over the whole body except the region just behind the operculum (fig. 1).

Here the color was olivaceous like that of a female, although not noticeably dissimilar from the condition found in many males. The characteristic short vas deferens ending at the

anal fin, the contact organs, and the dark spot at the base of the last rays of the dorsal fin, all indicated male sex. Upon examination of the internal organs, however, it was easy to discover ovarian traces (fig. 2). The testes were perfectly normal in position and general appearance and likewise differed in no respect microscopically. There were a large number of diffuse egg masses attached to the stomach and intestine and studding the mesentery binding these organs. The eggs were extremely small and immature. Apparently all the mature eggs had been expressed before the internal organs were exposed. The failure of the eggs to appear at the first trial pressure is readily ex-



Fig. 2 The internal organs of the hermaphrodite showing *L*, the liver; *S*, the stomach; *I*, the intestine; *T*, the testes; and a large number of immature eggs. The mature eggs were extruded when the supposed male was stripped.

plainable on the ground that a little violence sufficed to break the wall of the vas deferens and allow them to pass out through the male genital aperture. It is significant that there was no indication of an ovotestis, and that all eggs visible with the binocular were closely apposed to the organs of digestion.

Sections of the testes and of the whole digestive tract with organs undisturbed from their original relations have been recently made. It was found that not only did the eggs lie closely attached to the stomach and intestines, but that they extended in small groups under the serous coats of these organs and formed nests in the muscular coats (fig. 3). It was also extremely interesting to discover that a large part of the distal end of the left side of the liver was occupied by a group of immature eggs, surrounded by liver tissue and entirely hidden from the outside.



Fig. 3 Photomicrograph of a portion of the liver and stomach of the hermaphrodite fish showing eggs. (Photograph by Mr. F. H. Dodge.)

LITERATURE AND GENERAL CONSIDERATIONS

Although no case on record is like the one here described in the absolute independence of the gonadal substance, it will perhaps prove an advantage to briefly describe other cases of hermaphroditism in fishes.

Stephan ('01) describes a condition in *Sargus vulgaris*, such that there were young males with a trace of eggs in the gonads; young females with a bit of indifferent tissue representing testis; and true hermaphrodites. In regard to the production of true

males, true females and true hermaphrodites as age increased, he says:

L'age efface ces traces pour ne laisser subsister que le sexe predominant, sauf lorsqu'il y a à peu près equilibre; alors on a affaire aux hermaphrodites. . . . On remarque chez ce Poisson une grande plasticite des elements genitaux, qui fait qu'ils peuvent prendre une mauvaise direction de developpement et degenerer, ou bien remplir un rôle de secretion interne ou externe, ou quelque fonction. La plasticite et l'independance des divers elements sont le facteur principal qui permet d'expliquer l'apparition de l'hermaphroditisme dans des groups aussi eleves en organization.

Stephan also described certain species in which the males were precociously sexual and the females were late in maturing.

Roule ('02) found that all small individuals of certain Cyprinidae were males and all large individuals were females. He concluded that the individuals were protandric, being male when young and female when older.

Southwell ('02) described the roe of a smoked herring consisting of two parts, the anterior portion 95 mm. in length, being divided transversely into two lobes, and presenting the appearance of normal roe; while the posterior portion, lying between the two lobes of the roe in a wedge shape, was apparently normal testis 35 mm. in length. He also cites a case recorded by Smith ('70) in which the testis occupied the anterior region and the ovary the posterior region of the combined organ, and another specimen described by Mr. Yarrell in which there were two distinct lobes, one female and one male. No mention of the concomitant secondary sex characters of the specimens is included in the descriptions.

Newman ('08) has described a most interesting case of hermaphroditism in *Fundulus majalis* in which the secondary sex characters changed from female to some of those of the male during a period of one month. The external genital tube was oviducal, and the gonad was composed of about 5 per cent of testicular tissue, the rest being ovarian. The behavior changed from female to male also. About ten per cent of the eggs developed when fertilized.

Grassi ('11) described a male eel with several oocytes attached to the gonad.

Fowler (12) examined several hermaphrodite shad which had an ovotestis.

Okkelberg ('14) reported the existence in the brook lamprey, *Entosphenus wilderi* of a juvenile hermaphroditic condition normally. From a study of fifty individuals he found that 46 per cent were true females, 10 per cent were true males and 44 per cent were true hermaphrodites. Fifteen adult males were examined and of these seven contained undeveloped ova attached to the testes. The conclusion is that all the hermaphrodites develop into males.

The specimen of hermaphroditism here described is quite different from any of those hitherto recorded. It has diffuse egg masses extending from the stomach to the posterior portion of the large intestine, yet no single egg mass occurs nearer than the opposite side of the intestine to the testes. The suppression of further ovarian growth in other cases recorded is quite easy as the testis and ovarian substances are in close apposition. The wide distribution of the egg masses in this case is probably due to some environmental distribution of the anlage.

Persistence of the eggs even to apparent maturity, is rather difficult to explain if we attribute to the fish the same regulatory power possessed by the gonad of the mammal.

It is very evident on the other hand that an appreciably large per cent of ovarian tissue scattered through the body of a fish has no power to influence the formation of the secondary sex characters. In other cases recorded, notably the one described by Newman ('08) the presence of gonadal substance of the opposite sex in very small quantity, but in connection with the dominant gonad, was sufficient to prevent the full assumption of secondary sex characters. Any explanation of the condition we have described must recognize the evidence for an internal secretion produced by a regularly formed organ but absent in diffuse ovarian masses.

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REFERENCE MODEL OF THE THORACIC VISCERA

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THREE FIGURES

Recently a model of the abdominal viscera (Bellevue Model No. I) made by casting directly from a formalin hardened subject was described in the *Record* (vol. 10, p. 591). Adopting the same procedure, casts were made from the thorax and part of the neck of another subject. In the present case the subject used was a well-developed female who died of double lobar pneumonia, involving the lower lobes, at the age of thirty-four. The lungs showed no evidence of antecedent disease. There were a few soft pleuritic adhesions from the pneumonia of which the subject died. These were very slight and scarcely modified the natural surface of the pleurae.

The model consists of the lungs and of a stand comprising the cupolae of the diaphragm, the dorsal wall and diaphragmatic part of the pericardium, the lower part of the oesophagus, the larynx, trachea, bronchi, thymus and thyroid.

As in the abdominal model, the casts are made of a light plaster composition. The model complete is now sold by the maker uncolored but it is expected that casting in colors will soon be possible. In this case the colors, of course, will be permanent and will not be lost through wear, as in a painted model.

The model is represented in figures 1, 2, and 3, reduced about one-half. Figure 1 shows the medial surfaces of both lungs; figure 2, the entire model, posterior view; figure 3, the stand, anterior view. The following notes will explain the topography and anomalies of the dissection.

Lungs. The lungs show all the usual impressions on the mediastinal surface with the exception of that for the oesophagus near the base of the left lung. In the hilum, the cut sur-

E. V. Morrill

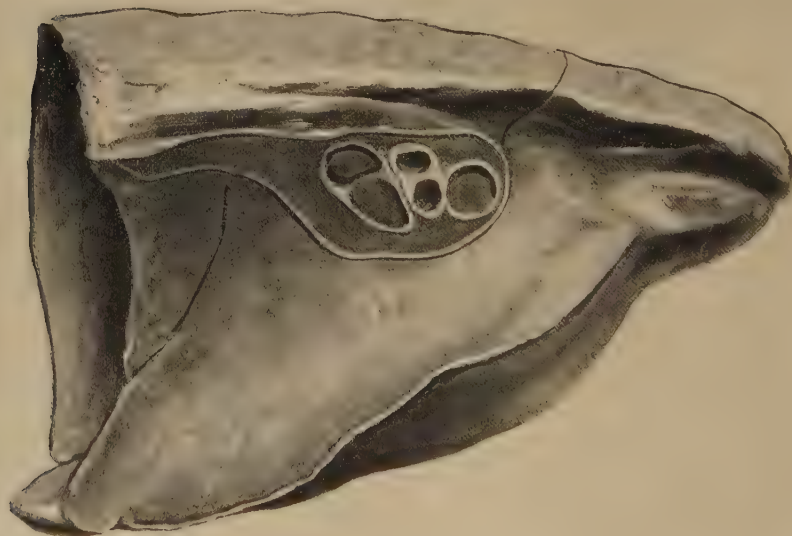


Fig. 1

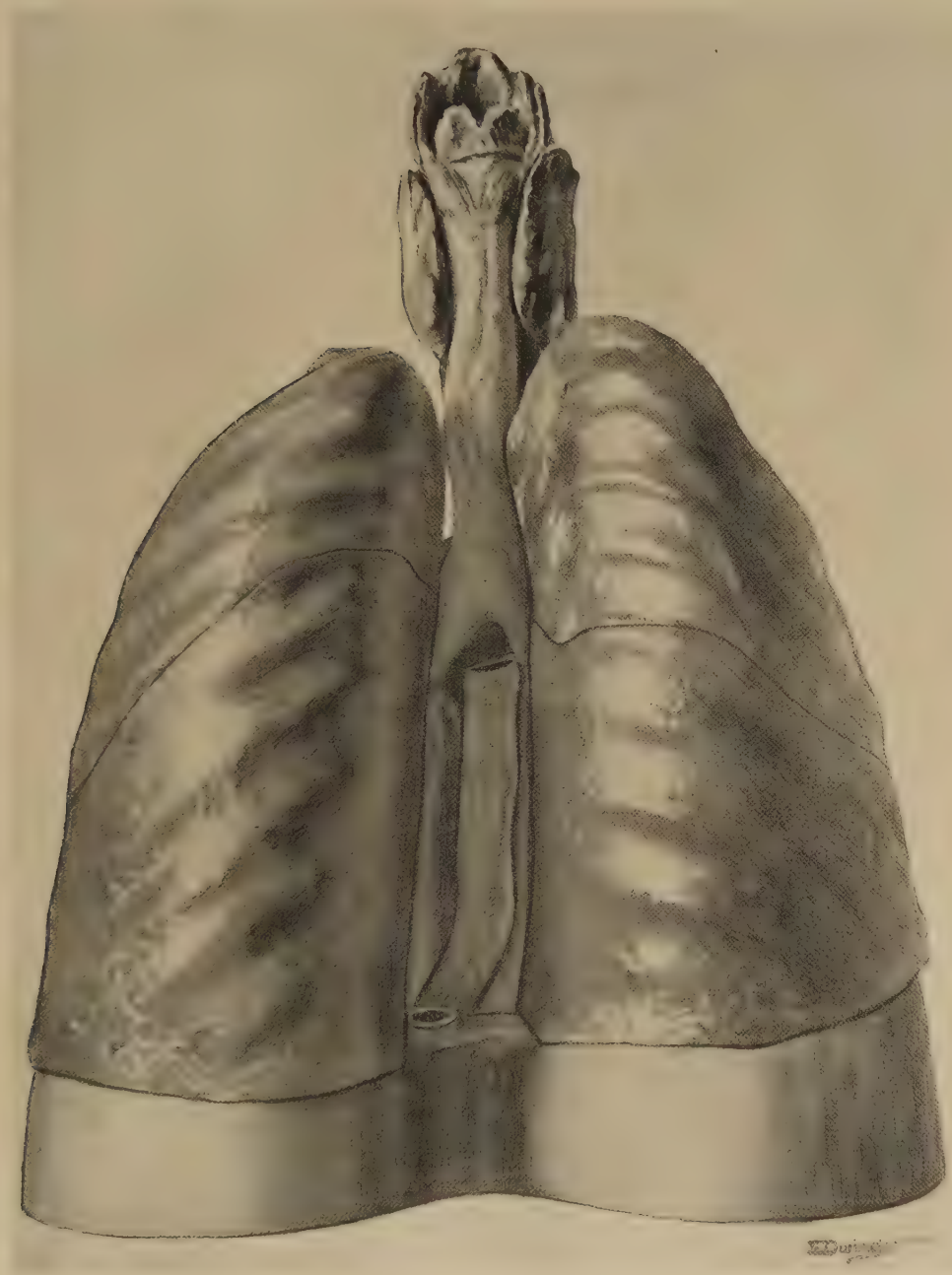


Fig. 2



Fig. 3

faces of the pulmonary arteries and veins and the bronchi are clearly marked. In the right lung the bronchus is dividing into eparterial and hyparterial parts, the latter giving off a branch which passes downward and forward. A right bronchial artery is shown passing to the posterior surface of the bronchus. The main stem of the pulmonary artery lies in front of the hyparterial bronchus while above it is the branch accompanying the eparterial bronchus. This branch is already subdividing into several smaller branches. The upper right and lower right pulmonary veins are in the usual positions, the former already dividing. In the hilum of the left lung the pulmonary artery, the bronchus and the pulmonary veins lie in the order named from above downward. The veins are uniting to form a common left pulmonary vein before entering the pericardium as shown in the stand. The left bronchial arteries, cut short, appear one above and one below the posterior part of the bronchus.

The ridges and furrows corresponding to the bony and muscular parietes are possibly accentuated by inflammatory swelling. These are unusually distinct. Some of the smaller vessels and nerves have left more or less distinct furrows upon the lung surface; thus there are impressions corresponding to the sympathetic cord and ganglia, the splanchnic and phrenic nerves and the internal mammary artery.

With regard to the furrows upon the costal surfaces of the lungs: The axes of these in the ventral region correspond to the main longitudinal axes of the ribs. As the furrows are traced dorsally, they may correspond either to the margin of a rib or to the internal intercostal muscle which frequently bulges beyond the level of the contiguous ribs. In order to map out upon the lung the areas occupied by the costal arches, it has been necessary to place the thoracic wall accurately in position upon the cast and then to mark accordingly. An old fracture of the right third, fourth and fifth ribs, in which union has occurred without accurate apposition of the bones, has left its impression upon the corresponding lung.

The stand. The dorsal portion of the pericardium remains in situ upon the diaphragm which forms the base of the model. In removing the heart, the vessels have been cut in such a manner as to indicate the lines of reflection of the serous pericardium around them. The left pulmonary vein is a single vessel at its point of entrance into the pericardium. The greater part of the aortic arch and descending thoracic aorta have been removed. The space occupied by these is clearly indicated when the left lung is placed on the stand. In the interior of the pericardium, the oesophagus and descending aorta have produced a broad vertical ridge between the lower right and common left pulmonary veins. The left portion of this ridge which corresponds to the aorta, is the more prominent and extends further upward.

The thymus gland was long enough to overhang the cephalic end of the pericardium. In order to reduce the complexity in this region, the right and left lobes of the gland were folded upward and secured in position before casting.

The pharyngeal mucosa has been left intact over the dorsal aspect of the arytaenoids and over the ventral and dorsal aspects of the upper part of the epiglottis. The dorsal aspect of the cricoid is partially covered by the posterior crico-arytaenoid muscles.

The model was prepared under the direction of Prof. H. D. Senior and the writer, Department of Anatomy, University and Bellevue Hospital Medical College. It is called "Bellevue Model No. II, A." All inquiries as to price, etc., should be addressed to Professor Senior. Orders may be addressed to G. Von Bouchaute, care of the above department.

NOTES ON THE PREPARATION OF BONES FROM MADDER-FED ANIMALS

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Past work upon bones of growing animals which have been subjected to a short course of madder-feeding has been done principally with dissected dried specimens and sections prepared by grinding and polishing thin slips. Decalcification, of course, decolorizes the dye, so that satisfactory sections cannot be made in the ordinary way. During some recent investigations with these vitally stained bones recourse was had to several methods which were found to be helpful in bringing to light the morphological details.

Of these methods perhaps the most useful was the familiar one of Spalteholz ('14), recently mentioned in this journal by Shipley and Macklin ('16) in connection with the study of bones vitally stained with trypan blue, and by Noback ('16) for the clearing of preparations of stained cartilage. Its advantages for the study of madder-bones are the sharpness with which the most rapidly growing areas of the bone are marked out, the clearness of the bony structure, and the ready means afforded of looking into the depths of the osseous tissue as well as upon the surface.

The technique is very simple: the specimens, fixed in 10 per cent neutral formalin, washed and cleaned, are dehydrated thoroughly in alcohol and treated with benzene followed by oil of wintergreen. Small bones, such as those of the rat, are particularly well suited to this method of treatment. In these, with the aid of the binocular microscope, the finer structure of the bone can be seen. The larger bones may be cut or sawn into thin sections before being cleared. In these the details are very distinct.

In bones from growing animals which have been fed with madder for a short time the areas of most rapid growth, as the ventral ends of the ribs, or the epiphysial lines of the long bones, are seen to be most densely stained. The region close to calcifying cartilage, where new spicules of bone are being formed, shows a fine velvet-like surface, while farther back on the shaft the trabeculae become coarser and the structure more open and plexiform. A cylinder of red, denoting the new bone formed in the process of periosteal ossification, characterizes the shaft of the bone. The stained centers of ossification are clearly outlined and their structure may be studied.

Membrane bones are strikingly shown by this method, the suture lines presenting a serrated edge of more densely stained bone, the tooth-like processes being quite red. The dye-deposits follow largely the lines of the blood vessels. The central cores of the teeth are well stained, but the enamel remains uncolored.

Beautiful preparations may be made of the bones of animals subjected to prolonged feeding with madder. A number of rats were fed in this way continuously from birth for five months (the dye being given at first in the mother's milk, with the result that the bones were colored throughout, as was to be expected, since all the calcium-salts laid down during the extra-uterine lifetime of the animal are impregnated with the dyestuff.

Sharp contrasts are presented where the normal feeding had been resumed for a few days, following a short term of madder-feeding, in a growing animal. Here the red bone stands out very clearly against the white bone which is deposited last, and which now caps the growing ends and lines the edges of the bones. Under higher magnification the relations of the colorless to colored bone in the trabeculae may be studied. Striking pictures of the way in which the bone grows and is absorbed are thus afforded.

Madder-stained pathological calcareous deposits may also be cleared by this method, and provide instructive specimens. The same is true for the callus formed in the repair of bone injury.

Permanent mounts in Canada balsam or damar may be made.

The Schultze method may also be used with good results in preparing these bones, and is especially to be recommended for entire specimens, where it is desired to retain the original outline. Embryos whose bones have been vitally stained by feeding madder to the mother have been successfully cleared in this way. The 1 per cent solution of KOH was employed, as recommended by Mall ('06). For the finer details of bone structure the Schultze method is not so good as that of Spalteholz.

In preparing dried specimens of madder-bones the method of maceration was employed, and was found to be very valuable in facilitating the cleaning of the skeletons. For the details of this method I am indebted to Miss Sara B. Conrow of The Wistar Institute, who uses it in the study of the unstained rat skeleton. The animal, having been skinned and eviscerated, is put into a solution of an alkali at a temperature of 95°C. to 97°C. and maintained at this temperature until the soft parts can be easily removed. A 0.2 per cent solution of KOH gave good results. Miss Conrow obtains splendid results with a hot 1 per cent or 2 per cent solution of ordinary 'Gold Dust' washing powder (a 2 per cent solution being used for adult rats). The young rat, after skinning and evisceration, is placed whole in the hot 1 per cent solution in the hot oven; with older rats, however, the larger masses of muscle are removed and a number of the bones disarticulated and placed in separate containers in the oven.

The length of time in the oven depends on the age of the rat and on how much muscle has been removed from the bones, though much more on the former. The young rat should be watched at intervals and the feet, when they soften, removed to shallow containers of tap water, to prevent their dropping off and the bones becoming mixed. The adult rat should be watched at the end of one hour in the oven and the condition of the flesh tested with a forceps. The soft parts should be so much softened that the bones will clean easily in tap water by the use of a small forceps, a tooth brush, a small bone scraper and perhaps a scissors.

If it is desired to maintain the numerous elements of the skeleton in their proper relationships one to another, the bones of the right and left sides of the body may be placed in separate containers in the oven and care taken in the cleaning to keep the bones in order.

The various bones, after being cleaned, may be laid out in their proper order on stiff cardboard and may afterward be mounted on glass plates if desired.

Bones prepared in this way are of a brilliant red color, the alkali serving to intensify the madder-dye.

The method of digestion, at 40°C. in a mixture of 1 per cent pepsin and 0.2 per cent HCl in distilled water, to which a little thymol was added to allay putrefaction, was tried, but was slower and the results were not so good as in the case of maceration in an alkaline solution, the effect of the acid being to decolorize the dye.

It is best to store the preparations in a dark place.

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A CULTURE MEDIUM FOR EUGLENA WITH NOTES ON THE BEHAVIOR OF EUGLENA

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I. INTRODUCTION

Quince seed jelly has been in common use as an agent for retarding the movements of Protozoans but as far as the writer can determine it has not been suggested as a culture medium.

While working with quince seed jelly to retard the action of the flagellum of *Euglena* it was discovered that *Euglena* would live for several days in the medium. *Paramoecium* also existed for several days in depression slides surrounded by the medium. It was conceived that Protozoans might be kept alive for a considerable length of time and an attempt to test this possibility suggested the value of further experimental work.

It was found that not only would *Euglena* live in the medium but it seemed to obtain nourishment from the medium and increased in great numbers. Cultures have been kept for fourteen months. Two hundred successful transplants have been made from a single culture and *Euglena* has been available for class work at all times throughout the year. The following experiments have been carried out to determine the best conditions for raising *Euglena*.

II. EXPERIMENTS TO DETERMINE THE OPTIMUM CONDITIONS FOR THE DEVELOPMENT OF EUGLENA

1. *Acidity and alkalinity*

Experiment a. Quince seed jelly was used which had been exposed to the air for several weeks and had acquired a strong acidity evidently through fermentation. Twenty-five tubes of the medium were prepared and inoculated with cultures of *Euglena* which had been grown on a neutral medium.

Six of the cultures contained live *Euglena* at the ends of three weeks. Two cultures contained *Euglena* at the end of six weeks and all were dead at the end of twelve weeks. Moulds were abundant in the cultures after five days. In three of the cultures a mould developed which contained a brilliant orange pigment.

Experiment b. Acid medium from the same stock as that used in the preceding experiment was neutralized with sodium carbonate.

Twenty tubes of the medium were inoculated with *Euglena* from the same culture as in the preceding experiment.

At the end of three weeks all the cultures were living and only three were dead at the end of six weeks. Sixteen of the twenty cultures were alive at the end of twelve and all had shown a great increase in the number of *Euglena*.

Experiment c. Twenty test tubes were prepared each containing 50 cc. of neutral medium. Five were kept as controls while amounts varying from one to seven drops of $\frac{N}{10}$ sodium carbonate were placed in the others. They were inoculated with *Euglena* from a neutral culture,

All cultures were alive at the end of two weeks but no marked difference in their progress could be distinguished. At the end of four weeks there was a slight difference in favor of the more alkaline cultures. After a period of seven weeks all the cultures were flourishing. There was no perceptible difference in cultures differing only slightly in their alkaline content but the most alkaline had progressed somewhat more than the neutral and the slightly alkaline ones.¹

Experiment d. The acid medium used in experiments a and b was used here. Fifty lots of media of 50 cc. each were prepared and arranged in ten equal groups. (The medium at this time had a total acidity which required 8 drops of $\frac{N}{10}$ sodium carbonate to neutralize 50 cc). Five cultures were kept on the acid medium as controls. Amounts of sodium carbonate varying from 3 to 17 drops were placed in the other lots. Group number one (the controls) were thus rather acid while group number ten was equally alkaline. Group number five was about neutral. *Euglena* from a 'Wild' culture were used to inoculate the tubes.

After six weeks active *Euglena* were found in the slightly acid, the neutral and all the alkaline cultures. In the alkaline cultures there had been a substantial increase in the numbers of individuals. No *Euglena* were found in the strongly acid cultures but a few encysted individuals were found in the cultures having a moderate acidity.

Experiment e. In cultures kept for a year in a thick jelly it was noticed that the medium was reduced to a thin liquid having the consistency of water wherever the animals had remained in one place for some time. This liquid was tested from time to time and gave a strong alkaline indication while the medium at the center of the tubes was still neutral. The liquid part of the cultures increased in its alkalinity with the age of the cultures. It is not necessary to postulate a specific substance in the decomposition products of *Euglena* to account for this liquefaction. A thick jelly may be reduced to a smooth liquid by ammonium hydrate or sodium carbonate and conversely a thin medium may be set to a jelly by a strong acid.¹

¹ In tests for alkalinity and acidity $\frac{N}{10}$ and $\frac{N}{50}$ solutions of sodium carbonate and of hydrochloric acid were used with alizarin as an indicator.

2. *Light*

Cultures kept in a moderate light were successful when other factors were equal. Cultures kept in the sunlight died in a short time. *Euglena* will not live in the dark.

3. *Density*

Experiment a. Two cultures were started in the fall of 1915 in very thick jelly. No effort was made beyond a preliminary boiling to keep the tube sterile. About five hundred individuals were placed in each culture. At the end of three months each culture showed a conspicuous green color at the top. After six months the green color was present at the tops, sides and bottoms. The medium at the top, sides and bottom was thin while that at the center of the tubes was unchanged. At the end of nine months about a third of the entire volume of the medium had been liquefied and this zone was occupied by enormous numbers of *Euglena*. Over two hundred transplants were made from one of the cultures at this time. At the end of the thirteen months both cultures had shown a still greater increase in numbers. The liquid zone had increased and as previously stated gave a strong alkaline reaction, whereas the original medium had been neutral.

Experiment b. Forty cultures were started under the same conditions as in experiment a.

After five weeks twenty-eight of the cultures had started through the course just described in experiment a. Twelve were dead and a thick growth of mould had taken possession of them. Most of the dead cultures displayed a slight acid reaction.

Experiment c. Media was prepared by evaporating the jelly to dryness and making up solutions to the desired consistency with distilled water. The following dilutions were made up: 0.75 per cent, 0.5 per cent, 0.4 per cent, 0.3 per cent, 0.25 per cent, 0.2 per cent, 0.1 per cent, 0.05 per cent, 0.025 per cent. Forty cultures were started with *Euglena* from a 'Wild' culture, five cultures upon each of the above named dilutions. Test tubes having a bore of 8.25 mm. were used.

At the end of a few weeks a few inactive *Euglena* were found in the cultures presenting a 1 and a 0.75 per cent dilution. A growth of mould had started at the tops of some of the cultures. In all the other cultures there was a downward migration, all the *Euglena* being arranged in a horizontal plane not more than 0.5 mm. in width. The extent of this downward migration varied directly in proportion to the density of the medium.

Dilution per cent	Vertical distance of migration mm.
0.5	2.8
0.4	3.25
0.3	3.6
0.2	4.25
0.1	8.0
0.05	11.0
0.025	17.0

At the end of two months only a few slowly moving and encysted individuals were found in the cultures representing a 1 per cent, a 0.75 per cent and a 0.5 per cent solution. In the others the migration had reached the bottoms of the tubes and there had been a great increase in the actual number of *Euglena*.

Heat and cold

Sixty cultures were prepared, twenty being in an incubator at 37°C., another twenty kept at room temperature and the rest in a temperature of about 7°C. Those kept at room temperature produced flourishing cultures. The increased heat of the incubator tended to promote bacterial growth and to dry up the medium. Those kept at 7°C. were slow to develop but produced good cultures when returned to room temperature. The lower temperature was favorable for the growth of mould.

5. Shape of receptacle

Cultures made the most rapid progress in test tubes, vials and jars where the *Euglena* had a considerable vertical range. Cultures were also tried in flat bacteria dishes where there was a large surface exposed.

These cultures were generally failures.

6. Sterile cultures

About 70 per cent of all cultures attempted with no precautions as to their being sterile were successful. Sterile media can be easily prepared, however, by boiling and cultures planted in sterile media give more uniform results. It is very difficult to keep cultures sterile as the introduction of *Euglena* can hardly be accomplished without giving access also to some bacteria.

7. Bacteria and moulds

The presence of a small quantity of bacteria does not seem to materially affect the progress of a culture except when the cultures are kept at a temperature of more than 21°C. The presence of moulds not only retards the progress of the culture, but the mycelium makes it difficult to follow the progress of *Euglena*.

Cultures containing a dense medium were found to contain a thicker growth of mould than those grown on a thinner medium. Experiments proved that a medium representing a 0.25 per cent solution of dried jelly in distilled water will not support a growth of mould. Neutral and slightly acid cultures usually contained growths of mould but the alkaline cultures, whether weak or strongly alkaline were free from it. Old cultures of *Euglena*, grown on a thick medium, will automatically clear themselves of mould by becoming alkaline.

8. *Effects of transplanting*

No weakening effects have been observed in transplanting cultures and frequently a slowly developing culture in which there is a tendency for the *Euglena* to encyst will become rejuvenated by being transplanted.

9. *Media prepared from ground seed*

A tendency to group about pieces of seed was exhibited by *Euglena* in some early cultures. An effort was made to test this tendency to see whether it might be of practical use. Seed was ground to a fine meal and a medium prepared by adding water in the proportion of 20 grams of ground seed to 2.5 liters of water. This medium produced the most favorable results for the rapid development of a culture. Fifty cultures of 50 cc, each were started, using this medium. About a thousand *Euglena* were placed in each culture. At the end of six weeks the numbers had increased till the entire volume of the cultures had assumed a bright green color and it was estimated that each culture contained 15,000 individuals per cubic centimeter.²

III. NOTES ON THE BEHAVIOR OF EUGLENA

The usual positive phototropism in exhibited *Euglena* shows a marked difference in its behavior in thick and thin media. Whatever the density of the medium there is a persistent effort to migrate downward. In thin jelly locomotion by means of the flagellum is possible and the downward migration is accomplished by this means. During this migration all the animals are to be found in a narrow horizontal zone as described under 'density', experiment three. In a very dense medium locomotion can take place only by means of 'euglenoid' motion and this is relatively ineffectual for progress unless the animal is in contact with a solid surface. The behavior described under 'density', experiment one can be explained according to this observation. Here, only the tops, sides and bottoms of the tubes are occupied or only those regions which can be penetrated by euglenoid locomotion.

² In estimating numbers a known quantity of media with the *Euglena* was diluted to a known volume. The tube was thoroughly shaken to insure an even distribution of the animals. A small measured amount of the medium was then placed under a binocular and the *Euglena* counted.

The density of the medium thus plays an important rôle in the behavior of *Euglena*, both in determining the mode of locomotion (whether euglenoid or flagellate) and second, in limiting the distance of migration within a given time.

IV. CULTURES OF OTHER ANIMALS IN QUINCE SEED JELLY

A minute green flagellate appeared in some of the *Euglena* cultures which had been inoculated from 'Wild' cultures. Transplants have been made and pure cultures obtained. So far these cultures have lived for four months and a half and are reproducing rapidly.

A large spiral shaped Bacterium was found to reproduce in great numbers in a slightly acid medium that had been kept at room temperature.

Paramoecium and a large Stylonychian have been kept for three months in mixed cultures. They feed upon *Euglena* and the bacteria.

Two small species of Rhizopods appeared in some mixed cultures and lived for about four weeks. An attempt was made to raise *Amoeba Proteus* in the medium but without success.

Nematode worms have been kept alive for a period of six weeks in the medium.

Mosquito larvae lived for three weeks in a rather dormant condition.

The medium seems to serve as food for the chlorophyll-bearing Protozoans and for some bacteria. The other animals live only as long as food is furnished in the form of *Euglena* and bacteria.

V. FLAX SEED JELLY AS A SUBSTITUTE FOR QUINCE SEED JELLY

A jelly may be obtained from flax seed by boiling the whole seed in water for a short time. At the date of this writing cultures of *Euglena* have been kept for five weeks in this medium and seem to be thriving quite as well as those in the quince seed jelly.

VI. PREPARATION

1. Quince seed jelly may be prepared quickly for laboratory use in the following manner:

Boil 20 grams of dry quince seed in 1.5 liters of distilled water for about half an hour, stirring occasionally. A gelatinous exudate will be obtained from the seeds. Pass the water containing the exudate through a wire sieve to remove particles of seed. (An Eimer and Amend sieve number 80 is fine enough to clarify the jelly and still permit it to pass through freely). Make up the volume to two and a half liters with distilled water. The medium will keep for several months if sterilized.

2. It is possible to obtain for experimental purposes a medium having a constant as regards density and chemical content by evaporating the jelly to dryness and making up standard dilutions with distilled water.

3. *Euglena* have been found to develop most rapidly on the following medium: ground quince seed, 20 grams; distilled water, 3 liters.

The above methods are practical for the laboratory because they require no special technique for their preparation and the cultures need but little attention when once started.

VII. SUMMARY

1. *Euglena* have been kept for over a year on a medium composed of quince seed jelly and water..

2. For cultures expected to last for a long time a thick jelly which has been rendered slightly alkaline should be used. Cultures planted on such a medium develop slowly.

3. Cultures develop more rapidly on a thinner medium.

4. Cultures develop most rapidly on a medium composed of ground quince seed and water. This medium has the disadvantage of being opaque.

5. Cultures should be kept at room temperature, in an moderate light and in glass containers having a considerable vertical range.

6. Other Protozoans have been kept for several months in the medium.

7. The behavior of *Euglena* is modified by the medium.

8. Transplants can be made successfully.

